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VOL. 30 AUGUST, 1952 NUMBER 4

OCCURRENCE OF AMINO ACIDS IN THE HONEYDEW OF THE CRESCENT-MARKED LILY APHID, *MYZUS CIRCUMFLEXUS* (BUCK.)[1]

BY J. B. MALTAIS[2] AND JACQUES L. AUCLAIR[3]

Abstract

The honeydew of *Myzus circumflexus* feeding on Katahdin potato contains 22 free amino acids and amides, which are given in their respective amounts. Carbohydrates constitute also an important portion of this product of digestion in aphids. Analyses of amino compounds in honeydew may be of prime importance in the study of the causes of plant resistance to aphids.

It has long been recognized that the honeydew of insects contains much unabsorbed organic matter, especially carbohydrates. The honeydew excreted by *Drepanosiphum platanoides* (Schr.) contains 55.44% sucrose, 24.75% invert sugar, and 19.18% dextrin (4). Of *Lachnus muravensis* (Arnh.) feeding on larch, the honeydew contains 3.94% invert sugar, 53.36% of a mixture of sucrose and melizitose, 30.3% dextrin, and 3.4% proteins (1). A species of the genus *Pemphigus* feeding on various plants produces honeydew that contains 85% carbohydrates and 3% proteins. The carbohydrate fraction includes 16.7% sucrose, 24.5% invert sugar, and 30.4% dextrin (6, 7). Very little has been reported of the composition of the protein fraction or of the occurrence of free amino acids in honeydew. Studies were made on the biochemical composition of the products of digestion in aphids as a possible clue to the factors responsible for plant resistance. This paper records qualitative and quantitative analyses of the free amino acids occurring in the honeydew of the crescent-marked lily aphid, *Myzus circumflexus* (Buck.).

The technique developed for the collection of the honeydew and its preparation for the biochemical and paper partition chromatographic analyses of its soluble constituents was as follows: potato plants, variety Katahdin, were grown in 6-in. flower pots in the laboratory near adequate window light during April and May. The plants were infested with apterous forms of *M. circumflexus* and the aphid population was allowed to develop under normal indoor conditions. The honeydew was collected on pieces of window glass placed

[1] *Manuscript received February 26, 1952.*

Contribution No. 2942, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Can. Presented at the first annual meeting of the Entomological Society of Canada and the eighty-eighth annual meeting of the Entomological Society of Ontario, Ottawa, November 1-3, 1951.

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close to the infested areas of the plants. After the pieces of glass were covered with a very apparent coating of honeydew, they were carefully washed with water; the washings were centrifuged and the clear solution was evaporated to dryness at mild heat with a light air jet under an infrared lamp. The residue was placed in vacuum for 48 hr. and weighed. It was later redissolved in a known quantity of slightly phenolized water to give a concentrated extract of 16.2 mgm. in 1 ml. The Kjeldahl (5) procedure for total nitrogen, the Van Slyke (8) nitrous acid method for amino nitrogen, and the Fehling (2) reaction for total sugars were made on the soluble constituents of the honeydew extract. The results were as follows: total nitrogen, 2.44%; amino nitrogen, 23.31 mgm. in 100 ml.; and sugars, 35.7%. These analyses indicate that in *M. circumflexus* feeding on potato plants the honeydew is composed of carbohydrates and a substantial amount of nitrogenous constituents such as amino acids and amides. Protein precipitants such as neutral lead acetate and ethanol caused a slight precipitate in a solution of the honeydew. This indicates that the honeydew contains also some proteins.

TABLE I

QUANTITATIVE PAPER CHROMATOGRAPHIC ESTIMATION OF THE FREE AMINO ACIDS CONTAINED IN HONEYDEW OF *M. circumflexus*

Amino acids and amides	I* mgm./100 ml.	II μgm./mgm. dry matter	III amino N, mgm./100 ml.	IV total N mgm./100 mgm. dry matter
α-Alanine	1.25	0.77	0.196	0.012
β-Alanine	0.25	0.15	0.039	0.002
α-Amino-*n*-butyric acid	0.3	0.2	0.041	0.002
Arginine	30.0	18.0	2.41	0.596
Asparagine	60.0	37.0	6.36	0.785
Aspartic acid	20.0	12.0	2.10	0.130
Cysteic acid**	0.44	0.27	0.051	0.003
Glutamic acid	20.0	12.0	1.90	0.118
Glutamine	30.0	18.0	2.86	0.352
Glycine	0.85	0.52	0.158	0.010
Histidine	10.0	6.2	0.902	0.167
Leucine and/or isoleucine	4.54	2.80	0.484	0.030
Lysine	6.6	4.1	0.632	0.078
Methionine sulphoxide	3.42	2.11	0.290	0.018
Phenylalanine	3.33	2.05	0.282	0.017
Proline	1.5	0.93	0.000	0.011
Serine	7.0	4.0	0.933	0.058
Threonine	3.75	2.31	0.441	0.027
Tryptophan	8.8	5.4	0.603	0.074
Tyrosine	10.0	6.2	0.773	0.048
Valine	2.4	1.5	0.287	0.018
Total	224.43	136.51	21.742	2.556

* *The results in Column I were obtained by quantitative paper chromatography. The results in Columns II, III, and IV were calculated from those of Column I.*

** *A few honeydew samples were treated with H_2O_2 to oxidize cystine or cysteine into stable cysteic acid.*

Qualitative and quantitative paper chromatographic analyses of the free amino acids in the honeydew were made by a method recently developed by Auclair and Dubreuil (3). The results are given in Table I.

Twenty-two amino acids and amides were identified in the honeydew of *M. circumflexus*. This total includes 21 amino compounds already listed in Table I, together with gamma-aminobutyric acid, the identification of which will be described in a subsequent report. The quantitative estimation as given by the quantitative paper chromatography method (3) amounts to 224.43 mgm. of amino acids in 100 ml. of honeydew extract, or 136.5 μgm. of amino acids in 1 mgm. of dry matter. In other words, on a dry matter basis, the honeydew contains 13.2% amino acids as against 35.7% invert sugar.

The total amino nitrogen given in Table I is 21.74 mgm. in 100 ml. This total, calculated from the results obtained by quantitative paper chromatography, agrees rather well with the total of 23.31 mgm. in 100 ml. obtained with the Van Slyke gasometric method. Similar agreement in results was obtained with the Kjeldahl total nitrogen, or 2.44% as against 2.55% in Table I.

Concurrent analyses of the amino acids of honeydew and juice extracts of the host plant would probably throw considerable light on the intermediary metabolism of amino acids in aphids. It is anticipated that such analyses will be of prime importance in the study of the causes of plant resistance to aphids.

Acknowledgment

The writers acknowledge the assistance of Miss B. Fortin in rearing and ensuring an adequate population of the insects for study.

References

1. Arnhardt, L. Osterreichischen Larchenhonigtau, Larchenmanna und Larchenhonig. Z. angew. Entomol. 12 : 457-472. 1927.
2. Association of Official Agricultural Chemists. Official and tentative methods of analysis. 6th ed. A. O. A. C. Washington, D.C. 1945.
3. Auclair, J. L. and Dubreuil, M. R. A simple ultramicromethod for the quantitative estimation of amino acids by paper partition chromatography. Can. J. Zool. 30 : 109-113. 1952.
4. Busgen, M. Der Honigtau. Biol. Zentr. 11 : 193-200. 1891.
5. Hengar Company. The Hengar technique for Kjeldahl procedure. Philadelphia, Pa. Trade pamphlet.
6. Toth, L. Uber die fruhembryonale Entwicklung der viviparen Aphiden. Z. Morphol. Oekol. Tiere, 27 : 692-731. 1933.
7. Toth, L. Entwicklungszyklus und Symbiose von *Pemphigus spirothacae* Pass. Z. Morphol. Oekol. Tiere, 33 : 412-437. 1937.
8. Van Slyke, D. D. The quantitative determination of aliphatic amino nitrogen in minute quantities. J. Biol. Chem. 16 : 121. 1913.

EFFECTS OF STARVATION ON LARVAE OF THE PALE WESTERN CUTWORM, *AGROTIS ORTHOGONIA* MORR. (LEPIDOPTERA: PHALAENIDAE)[1]

By L. A. Jacobson[2]

Abstract

All larval instars of the pale western cutworm, *Agrotis orthogonia* Morr., were starved for varying periods at 10°, 15°, 20°, and 25° C. Mortality curves indicated varying individual susceptibility or resistance to starvation. The time required for 100% mortality varied inversely with temperature and directly with size of larvae. The rate of mortality of newly hatched larvae whether fed or unfed was nearly the same at all temperatures. Application of the findings to recommended control practices is discussed.

Introduction

One of the control methods of the pale western cutworm, *Agrotis orthogonia* Morr., is based on starvation of the first-instar larvae by destroying early plant growth by cultivation. Although the effectiveness of this control is dependent upon the destruction of newly hatched larvae, it is possible that all larval stages may be subjected to varying degrees of starvation in the field. Occasionally, anticipated outbreaks fail to materialize, and starvation may be one of the factors in the rise and decline of outbreaks.

One of the first objectives in assessing starvation as a factor influencing population was to measure the mortalities from starvation of all stages of larvae at four levels of constant temperature.

Methods

In order to have a uniform source of material for these investigations, the larvae were all hatched from eggs deposited by field-collected females from the same locality. At first, hatched larvae were bulk-reared in 3-oz. salve tins containing about three-quarters of an inch of plaster of Paris to achieve suitable humidity and to aid in locating small larvae. For the later stages of the investigation, Petri dishes of varying size were used for bulk or individual rearing and were considered superior. Hitherto individual rearing was preferred but the bulking method made it possible to increase the number of larvae under observation.

When stages other than the first instar were required for starvation the larvae were obtained from a reservoir of bulk-reared material. On molting to the required instar each larva was placed in a separate container and daily records on starvation mortality were kept from that day.

Mortality from disease and cannibalism was extremely low when an adequate food supply was available. During the starvation period it was necessary to isolate larvae to overcome cannibalism. First- and second-instar

[1] *Manuscript received February 5, 1952.*
Contribution No. 2929, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Associate Entomologist, Field Crop Insect Laboratory, Lethbridge, Alta.*

larvae were placed singly in small plastic boxes and the more mature larvae in 1-oz. salve tins. The desired humidity was achieved in these containers by using moistened blotting paper.

The plaster of Paris in the 3-oz. salve tins and the blotting paper in the plastic boxes and in the 1-oz. salve tins were moistened at frequent intervals to maintain a high humidity and thus eliminate the effect of desiccation, which would influence the mortality.

Wheat sprouts, including the kernel, about 1½ in. long, were used for food.[1] The wheat was germinated between sheets of moistened paper towelling, arranged in several layers in flat, galvanized iron trays. Satisfactory growth was obtained at room temperature.

Constant temperatures of 10°, 15°, 20°, and 25° C. were chosen as representing the temperature range encountered in the field during the larval period. These temperatures were maintained in walk-in rooms where the daily variation was within 1° C. As far as possible, all observations were made within the room at the temperature to which the series was being subjected in order to avoid undue temperature fluctuations.

Each series consisted of 50 larvae, in five groups of 10, each group being considered a replicate. Check series at each temperature, consisting of a similar number of larvae, were fed throughout the experimental period.

Newly hatched larvae were starved and fed, or fed, starved, and fed, as follows:—

Starved for 5, 10, 15, or 20 days before feeding.
Fed for 5 days, then starved for 4, 8, or 12 days and fed again.
Fed for 10 days, then starved for 4, 8, or 12 days and fed again.

This was later modified to include starvation of all instars. It soon became obvious that comparison of mortality at the highest and the lowest temperature could not be made because of the differences in development at different temperatures.

After 10 days of feeding at 25° C. the larvae were in the second instar, whereas at 10° C. very little development was noted. For this reason, comparisons were finally made on the effect of starvation on larvae of equal development at each temperature.

Second-, third-, fourth-, fifth-, and sixth-instar larvae were starved at 25° C. and all stages except the sixth at 20° and 15° C. Sixth-instar larvae were not included at these two temperatures as most of them would pupate, thus masking the effect of starvation.

Results

Starvation of First-instar Larvae

The effect of starvation on first-instar larvae was studied to determine mortality rates at the four constant temperatures and to note differences, if

[1] *The method of feeding pale western cutworm larvae with wheat sprouts was developed by Prof. B. Hocking, Department of Entomology, University of Alberta, through a grant-in-aid provided by Science Service, Canada Department of Agriculture.*

any, in mortality between larvae that were starved after hatching and those fed before starving. The results of starvation, expressed graphically as mortality curves at constant temperatures of 10°, 15°, 20°, and 25° C., are shown in Fig. 1.

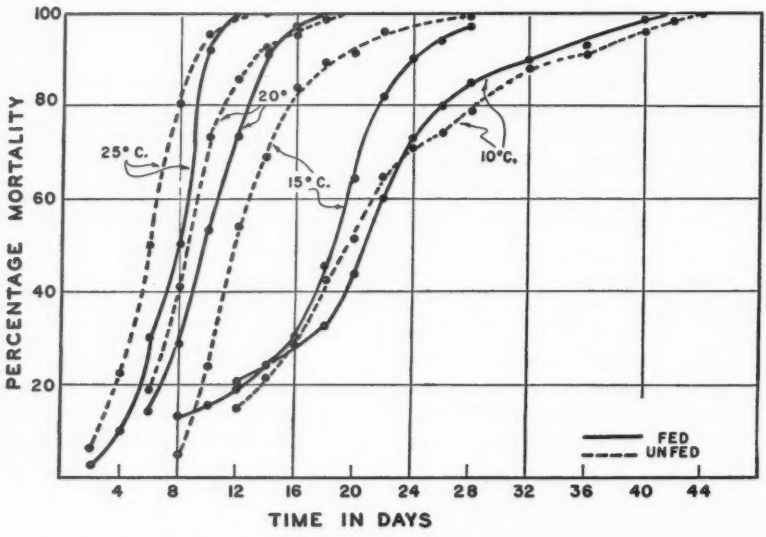

FIG. 1. Starvation mortalities of fed and unfed first-instar larvae of the pale western cutworm, *Agrotis orthogonia* Morr., at various constant temperatures.

The curves suggest that, at the lower temperatures, feeding before the larvae were starved may delay mortality from starvation in the early part of the starvation period. After the median point was reached some acceleration in mortality occurred among fed larvae. At each temperature the time required for 100% mortality of fed larvae was about the same as for the unfed. At the median point, however, there was a difference in mortality between the two series, as shown by the divergence of the curves. This was most evident at 15° C., whereas the difference at 10° C. was very small.

The data from which the curves in Fig. 1 were prepared were examined statistically by a method of group comparison described by Snedecor (5, p. 77). Analysis of the mean numbers of days for deaths of fed and unfed larvae showed a significant variance at 15° C. at the 5% level.

Percentage survival was dependent upon the temperature and length of the starvation period. At 25° C. mortality was 80 and 100% after starvation periods of 8 and 10 days, respectively. At 15° C. the mortality was about 90% after 20 days' starvation.

Fig. 2 shows the mortalities during and after 10 days' starvation after hatching. At 25° C. all the larvae died in 10 days. Survival was proportionate at the other temperatures.

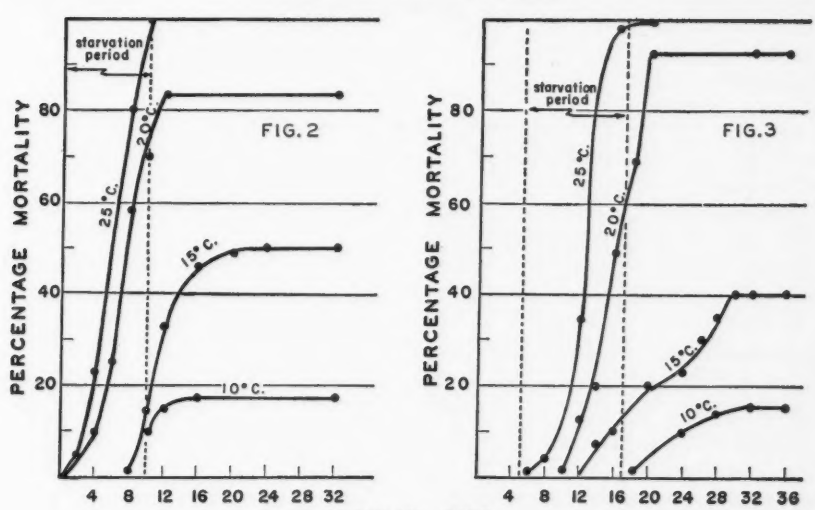

FIG. 2. Mortalities of first-instar larvae of the pale western cutworm starved at various temperatures for 10 days before feeding.

FIG. 3. Mortalities of first-instar larvae of the pale western cutworm fed for five days and starved at various temperatures for 12 days before feeding resumed.

When food was made available after starvation, the most striking effect was the cessation of mortality a day or two later. The mortality in the intervening period may be attributed to the fact that the larvae were too weak from starvation to resume feeding. The surviving population continued to develop normally with no further mortality.

Fig. 3 shows the mortalities of larvae starved for 12 days following five days' feeding after hatching. At 10° and 15° C. this starvation period had very little effect on mortality. At 20° and 25° C. mortality was directly proportional to temperature. In all instances the effect of starvation as measured by mortality continued after food was available, indicating that some of the larvae were incapable of feeding. Once this effect was overcome the survivors fed normally.

The results shown in Figs. 2 and 3 indicate that mortality from starvation varied directly with temperature and time. Further, at 25° C. feeding before starvation did not alter the time required for 100% mortality. Also, the rate of mortality at each temperature approximated the data shown in Fig. 1.

Mortality in the checks, from causes other than starvation, was negligible.

Starvation of All Instars

The results of starvation of all instars at 25° C. are shown in Fig. 4. There was a positive association between size of larvae, expressed as instars, and

longevity while undergoing starvation. One hundred per cent mortality of fifth- and sixth-instars was not achieved, as some of the larvae pupated. The pupae were small and, in some cases, malformed.

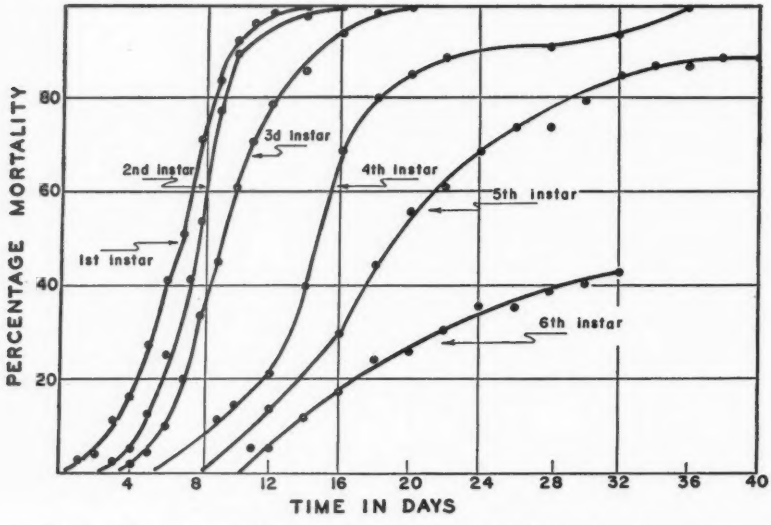

FIG. 4. Starvation mortality at 25° C. of each instar of the pale western cutworm.

Data on the times required for 50% mortality at 25°, 20°, and 15° C. are shown in Table I. These data indicate that mortality varied directly with temperature and inversely with size of larvae.

TABLE I

MEDIAN STARVATION MORTALITIES OF THIRD-, FOURTH-, AND FIFTH-INSTAR LARVAE OF THE PALE WESTERN CUTWORM AT 15°, 20°, AND 25° C.

Instar	Number of days for 50% mortality		
	15° C.	20° C.	25° C.
3	16	13	9
4	26	20	15
5	38	22	19

Discussion

The effect of starvation on all larval stages of the pale western cutworm is important, as it involves one of the recommended control principles for this insect. Its effect in the field may also be one of the factors limiting local or even regional outbreaks.

In the field it is possible that all stages of the larvae may be subjected to periods of critical food supply and even starvation. This may hold particularly for first-stage larvae, which hatch with the first disappearance of snow cover and some time before first plant growth.

The experiments described herein indicate individual variation in susceptibility or resistance to starvation, as shown by the mortality curves. The curves in Fig. 1 point to resistance after feeding, particularly at lower temperatures. The apparent resistance of those larvae fed before starvation may have been due to retention of food in the digestive tract. At 15° C. this was very evident and suggests that this temperature is a critical one in starvation and should be investigated further.

Seamans (2) and Seamans and Rock (4) referred to the ability of newly hatched larvae to withstand starvation. The latter stated that first-instar larvae can live for long periods without food, although Seamans (2) included the modification that this is true if conditions are cool. These findings were confirmed but it is very evident that the starvation period should be considered in terms of prevailing temperatures. It is possible that under a wide range of outdoor conditions the temperature of the soil inhabited by the larvae may be higher than suspected. Temperatures at or very near the soil surface are much higher during sunny periods than the temperature of the air some distance above the soil. Pale western cutworm eggs hatch early in the spring, and often considerable time elapses before grain seeding is general. If the weather is cool in this interval, mortality from lack of food may be negligible; but if warm, sunny weather occurs sufficient time may elapse to achieve a considerable reduction in the potential larval population.

In applying starvation control, the period of starvation as indicated by the time between the cultural plant-destroying operation and germination of the seeded crop should be modified in the light of prevailing temperatures. It is possible that the recommended period of 10 to 14 days may be more than adequate in warm weather and not sufficiently long in cool weather.

In the first instar, no marked difference was noted between fed and unfed larvae in the ability to resume feeding after starvation. Salt and Seamans (1) made the observation that a digestive abnormality was suspected after starvation, many of the larvae becoming bloated and unable to continue feeding. Such a condition was not observed in the course of these investigations. This may have been due to the use of wheat sprouts as food, the former workers having used clipped portions of the growing leaf. Differences in reaction and growth may be expected, therefore, when several parts of the same food plant are used. Differences between food plants, expressed as rates of development, were noted by Seamans and McMillan (3).

Hitherto, control recommendations utilizing starvation have been confined to the early stages. These data indicate that later stages, particularly the third and possibly the fourth instar, can be starved in the field if the starvation period is of sufficient length to assure mortality at the prevailing temperatures.

This would not always be possible, as too long a delay in the seeding date may limit the possibility of obtaining a harvestable crop.

When a crop has been completely destroyed by fourth- and fifth-instar larvae, re-seeding in compliance with present recommendations is not safe until the larvae have practically completed feeding. At this stage of the seasonal history the time required to destroy a population by starvation is too long to be considered for possible use in control recommendations.

There is a distinct possibility that cannibalism may further reduce the numbers of starving larvae, as the eggs are laid in groups and larvae are often in close association with each other.

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The assistance of Mr. R. R. Forster, Field Crop Insect Laboratory, Lethbridge, in maintaining food supplies and conducting many of the routine observations aided in the prosecution of these investigations. Miss R. I. Larson, of the same laboratory, assisted in the statistical analysis of data.

Acknowledgment is made to Mr. H. L. Seamans, Head, Field Crop Insect Investigations, Division of Entomology, Ottawa, for permission to use unpublished notes and reports prepared while he was in charge of pale western cutworm investigations at the Lethbridge Laboratory.

References

1. SALT, R. W. and SEAMANS, H. L. Experimental starvation of first instar larvae of pale western cutworm, *Agrotis orthogonia* Morr. Can. Entomologist, 77 : 150–155. 1945.
2. SEAMANS, H. L. A probable cultural control for the pale western cutworm, *Agrotis orthogonia* Morr. Sci. Agr. 17 : 612–615. 1937.
3. SEAMANS, H. L. and MCMILLAN, H. E. The effect of food plants on the development of pale western cutworm, *Agrotis orthogonia* Morr. J. Econ. Entomol. 28 : 421–425. 1935.
4. SEAMANS, H. L. and ROCK, P. J. G. Starvation of early instars of the pale western cutworm, *Agrotis orthogonia* Morr., and its use in the control of this pest. Can. Entomologist, 77 : 57–61. 1945.
5. SNEDECOR, G. W. Statistical methods. 4th ed. Iowa State College Press, Ames, Iowa. 1946.

SOME EFFECTS OF PROGESTERONE ON THE RESPONSE OF THE IMMATURE PULLET TO ESTROGEN, AND SOME OBSERVATIONS ON THE EFFECTS OF GONADAL HORMONES ON THE AVIAN SPLEEN AND KIDNEY[1]

By W. E. J. Phillips,[2] R. H. Common,[3] and W. A. Maw[4]

Abstract

Progesterone depressed the hypertrophy of the immature pullet's oviduct evoked by estrogen, but did not significantly affect the responses of liver weight, total liver crude protein, and serum calcium level evoked by estrogen. These effects are in some respects the converse of previously recorded effects of thiouracil on the response of the pullet to estrogen. Progesterone did not affect the increases of liver pentose nucleic acid and desoxypentose nucleic acid evoked by estrogen. Testosterone propionate by itself, at the levels used, did not affect the amounts or ratios of liver pentose nucleic acid and desoxypentose nucleic acid. Estrogen reduced spleen weight to a highly significant degree, and progesterone significantly reduced this effect of estrogen. Testosterone did not affect spleen weight. In the experiment described estrogen increased kidney weight to a highly significant degree; progesterone tended to reduce this effect, but the tendency did not attain significance at $P = 0.05$.

Introduction

The effects of estrogen on the sexually immature pullet may be modified by thyroxine (6, 2) and by thiouracil (4). Thiouracil depressed the increases of serum calcium, serum riboflavin, and serum vitamin A evoked by estrogen. In these respects the results of administering thiouracil resembled the effects of administering thyroxine. Thiouracil, however, enhanced the responses of oviduct, liver weight, and weight of liver crude protein to estrogen, whereas thyroxine depressed the responses of liver weight and of liver crude protein and did not significantly influence the response of the oviduct.

The steadily increasing significance attached to progesterone in relation to the avian reproductive cycle has been one of the most significant trends in avian endocrinology during the past decade. In 1943 Fraps *et al.* (8) demonstrated the presence of avidin in the functional magnum of the hen's oviduct. Shortly afterwards Hertz, Fraps, and Sebrell (10) showed that the hypertrophied oviducts induced in immature chickens by estrogen, or by estrogen plus testosterone, did not contain avidin. Administration of progesterone along with estrogen, however, did lead to the production of avidin in the oviduct. Fraps and Dury (7) made the further important observation that progesterone is highly effective in inducing ovulation in the fowl. Later work by Fraps *et al.* (9) demonstrated the presence of a progestogen in the plasma

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[3] *Professor of Agricultural Chemistry.*

[4] *Professor of Poultry Husbandry.*

of the laying hen. The significance of the induction of ovulation by progesterone in relation to normal ovulation remained obscure until observations on the fluctuations of plasma progestogen during the laying cycle in conjunction with a sustained series of observations on the time relations of ovulation and pituitary function enabled Rothchild and Fraps (13) to propound a plausible theory of the probable physiological sequence. These workers believed that release of ovulation hormone (LH) from the pituitary provides the immediate stimulus for ovulation, and that the release of LH is in turn stimulated by progestogen liberated from the follicle. If this theory were correct, it should be possible to increase the number of eggs in the clutch of laying birds by suitably timed injections of progesterone. Neher and Fraps (12) have demonstrated that it is possible to secure such increases. The foregoing studies provide strong evidence that progestogen plays essential roles in the physiology of reproduction by the hen.

The effects of progesterone on the response of the chick's oviduct to estrogen have been studied by Hertz *et al.* (11), who have shown that progesterone diminishes the hypertrophy evoked by estrogen. Their data, however, seem insufficient to settle whether progesterone modified the response of serum calcium to estrogenic treatments, and they did not report data for other organs or tissues.

The present paper deals with an experiment designed to examine more closely the effects of progesterone on the pullet's response to estrogenic treatment.

Experimental

General Technique and Analytical Methods

The pullets (New Hampshire ♂ × Barred Plymouth Rock ♀) were maintained in single cage laying batteries, and were assigned at random (*a*) to treatments and (*b*) to places in the batteries.

Each of the pullets received the same total amount of food. It is of the greatest importance to guard against possible effects of differences in appetite produced by hormonal treatments in experiments of this kind.

The hormones* were administered intramuscularly in oily solution, the treatments being given in 12 equal daily doses.

On the morning of the 13th day of experiment the birds were decapitated and bled. The livers were excised immediately, weighed rapidly on a torsion balance, and a portion of about 10 gm. was taken and disintegrated in ice-cold 10% trichloroacetic acid in the Waring Blendor, dry ice being added to maintain a low temperature. The weight of the sample taken was then obtained by difference. Samples were also taken for liver dry matter and crude protein.

The sample taken in the Waring Blendor was prepared for nucleic acid determinations as described previously (3). Pentose nucleic acid phosphorus (RNAP) and desoxypentose nucleic acid phosphorus (DNAP) were determined by the method of Schneider (14).

* *Estradiol benzoate as "Progynon B" Schering; testosterone propionate as "Oreton", Schering; progesterone as "Lutocyclin", Schering.*

Experimental Results

The pullets were 58 days old at the beginning of the experimental period. The treatments and the main experimental data are summarized in Table I.

The main inferences from the data presented in Table I are tabulated in Table II in order to avoid lengthy verbal description. Certain relevant findings of previous work (4) are similarly tabulated in Table III to facilitate comparison with the findings presently reported.

Effects of Progesterone on Responses of Oviduct, Liver, and Serum Calcium to Estrogen

From the standpoint of the immediate objective of the experiment, the salient feature of the results is that progesterone influences the responses of oviduct, liver, and serum calcium in a manner which is almost the converse of the influence of thiouracil on the responses of these organs to estrogen. Thiouracil enhanced the effect of estrogen on hypertrophy of the oviduct and depressed the effect of estrogen on serum calcium, whereas progesterone reduced the effect of estrogen on the oviduct, but did not influence the response of serum calcium.

It has been suggested (4) that the effect of thiouracil on the response of serum calcium to estrogen is not associated with interference with the increase of liver weight, and that it may be due to some kind of interference with the functioning of the hypertrophied liver. In the present experiment progesterone does not appear to have interfered with the functioning of the liver as regards phosphoprotein synthesis. It would seem unlikely that progesterone interferes with inactivation of estrogen in the liver. It might be suggested, tentatively, that the inhibiting effect of progesterone is exerted at the target organ (i.e. the oviduct).

It was noticed that the oviducts of the pullets receiving estrogen plus progesterone were structurally well differentiated, the degree of differentiation being greater than it would have been in an oviduct of the same weight produced by an appropriately smaller dose of estrogen.

None of the hormonal treatments affected the concentration of crude protein in the livers, but estrogen produced the expected large increase in total liver crude protein. Progesterone did not significantly modify this response.

Testosterone propionate did not affect either the concentration or total amounts of nucleic acids in the liver, in confirmation of previous results (3), with the exception that the increase in liver RNAP mgm./100 gm. was just significant at $P = 0.05$ when the "t" test was applied to the data for Groups 1 and 2. Estrogen produced the expected large increase in total liver RNAP and the data confirmed the effect of estrogen in producing a moderate increase of total liver DNAP. (This effect is being studied at present in greater detail by relating the increase to possible changes in numbers of liver nuclei or their content of DNAP.) It will be noted that progesterone did not in the slightest degree modify the responses of the liver nucleic acids to estrogen. In previous work (3) testosterone propionate was found not to affect the responses of liver

TABLE I

EFFECT OF PROGESTERONE ON CERTAIN RESPONSES OF THE SEXUALLY IMMATURE PULLET TO ESTROGEN

Group	1	2	3	4	Necessary diff. between group means	
No. of pullets treated	7	7	7	7	$P = 0.05$	$P = 0.01$
Total dosage estradiol benzoate, mgm.	Nil	Nil	12 × 1.0	12 × 1.0		
Total dosage testosterone propionate, mgm.	Nil	12 × 1.0	Nil	Nil		
Total dosage progesterone, mgm.	Nil	Nil	Nil	12 × 1.0		
Initial live weight, kgm.	0.67	0.65	0.66	0.68	NS	—
Final live weight, kgm.	0.96	0.97	0.93	0.92	NS	—
Live weight increase, kgm.	0.29	0.32	0.27	0.24	NS	—
Food consumption, kgm.	0.84	0.84	0.84	0.84	—	—
Ovary, gm.	0.28	0.23	0.24	0.23	NS	—
Oviduct, gm.	0.18	0.28	9.26	3.26	0.95	1.06
Serum calcium, mgm./100 ml.	11.7	11.4	71.3	72.3	See text	—
Liver, gm.	19.03	18.79	31.04	31.02	3.19	3.58
Liver, gm./kgm. live weight	19.95	19.53	33.38	34.00	3.66	4.11
Kidneys, gm.	7.13	5.84	8.02	8.69	0.713	0.800
Kidneys, gm./kgm. live weight	7.52	6.07	8.66	9.35	1.33	1.50
Spleen, gm.	1.63	1.69	0.97	1.43	0.345	0.388
Spleen, gm./kgm. live weight	1.69	1.79	1.05	1.55	0.383	0.430
Liver crude protein, %	18.4	18.3	18.8*	18.7*	NS	—
Liver crude protein, gm./kgm. live weight	3.61	3.49	5.97*	6.42*	0.85	0.96
Liver RNAP, mgm./100 gm.	71.0	75.7	86.0*	79.6*	NS	—
Liver RNAP, mgm./kgm. live weight	14.1	14.7	28.1*	28.0*	4.10	5.58
Liver DNAP, mgm./100 gm.	30.0	34.4	24.5*	22.8*	4.53	5.09
Liver DNAP, mgm./kgm. live weight	6.00	6.74	8.28*	8.00*	1.26	1.71
Ratio $\frac{\text{liver RNAP}}{\text{liver DNAP}}$	2.44	2.25	3.56*	3.54*	0.80	1.08

** Average for six pullets only. The necessary differences are slightly less as between Groups 1 and 2, and slightly greater as between Groups 3 and 4. It so happens that the group differences involved do not approach significance, except in the case of liver DNAP mgm./100 gm. which is discussed in the text.*

TABLE II

INFLUENCE OF PROGESTERONE (PGST) ON CERTAIN RESPONSES OF THE IMMATURE PULLET TO ESTROGEN: MAIN INFERENCES FROM THE DATA PRESENTED IN TABLE I

Response of	Hormonal treatment			Inference
	Testosterone propionate (TSTP)	Estradiol benzoate (ODB)	Estradiol benzoate plus progesterone (ODB + PGST)	
Oviduct weight	+ Small	+ Very big	+ Moderate	PGST antagonized effect of ODB
Liver weight	Nil	+ Big	+ Big, not different from that for ODB alone	PGST did not antagonize effect of ODB
Liver crude protein, gm./kgm. live weight	Nil	+ Big	+ Big, not different from that for ODB alone	PGST did not antagonize effect of ODB
Serum calcium	Nil	+ Big	+ Big, not different from that for ODB alone	PGST did not antagoniz effect of ODB
Kidney weight	− Considerable	+ Considerable	+ Bigger than with ODB alone	Kidney responded to TST and to ODB in different way to either liver or oviduct
Spleen weight*	Nil	− Big	− Slight	PGST antagonized effect of ODB
Liver RNAP, mgm./kgm. live weight	Nil	+	+ Not different from response for ODB alone	PGST did not antagnonize effect of ODB
Liver DNAP, mgm./kgm. live weight	Nil	+	+ Not different from response for ODB alone	PGST did not antagonize effect of ODB

** Similar effects of TST and ODB have been noted in several other unreported experiments.*

nucleic acids to estrogen at the levels used, although it may be that a significant increase would be observed using other levels.

Effects of Gonadal Hormones on Kidney Weight

Burrows (1) quotes various experimenters who have noted increases of kidney weight in rats or mice following treatment with androgen, and some of these experimenters have noted a similar increase of weight following administration of estrogen. It is, therefore, somewhat remarkable to note that testosterone propionate produced a highly significant decrease of kidney

TABLE III

INFLUENCE OF DIETARY THIOURACIL ON RESPONSES OF THE IMMATURE PULLET TO ESTROGEN (4). RESULTS PRESENTED FOR COMPARISON WITH RESULTS PRESENTED IN TABLE II

Response of	Hormonal treatment		Inference
	Estradiol benzoate ODB	Estradiol benzoate × dietary thiouracil	
Oviduct weight	+ Big	+ Bigger than with ODB alone	Thiouracil enhanced effect of ODB
Liver weight	+	+ Much bigger than with ODB alone	Thiouracil enhanced effect of ODB
Liver crude protein, gm./kgm. live weight	+	+ Much bigger than with ODB alone	Thiouracil enhanced effect of ODB
Serum calcium	+ Big	+ But small in comparison with that from ODB alone	Thiouracil antagonized effect of ODB

weight in the present experiment. The estrogenic treatment, on the other hand, caused a highly significant increase in kidney weight; progesterone tended to increase this response, but this effect did not attain significance at $P = 0.05$. The divergence from results secured in comparable experiments with mammals calls for further study. It may be remarked here that this effect of testosterone propionate on kidney weight has not been observed in another experiment (unpublished results) using somewhat smaller levels of androgen.

Effects of Gonadal Hormones on Spleen Weight

It has been pointed out previously that estrogen depresses spleen weight in the immature pullet (5). Selye (15) has also recorded a depressant effect of estrogen on spleen weight in rats. The observations reported in Table I provide ample confirmation of this effect in the bird. The results also demonstrate that the effect of testosterone on spleen weight was negligible, and that progesterone significantly reduced the effect of estrogen on spleen weight. It might be remarked here that a positive effect of heavy doses of folic acid on spleen weight has been observed in this laboratory (unpublished results) and it seems evident that the folic acid status of the bird may affect the response of its spleen to gonadal hormones.

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indebtedness to Messrs. Schering Corporation, Montreal, for their unfailing generosity in providing hormonal preparations. They also wish to place on record the invaluable aid freely given by their fellow students in this laboratory.

References

1. BURROWS, H. Biological actions of sex hormones, 2nd ed. Cambridge University Press, Cambridge. 1949.
2. COMMON, R. H., BOLTON, W., and RUTLEDGE, W. A. The influence of gonadal hormones on the composition of the blood and liver of the domestic fowl. J. Endocrinol. 5 : 263–273. 1948.
3. COMMON, R. H., CHAPMAN, D. G., and MAW, W. A. The effect of gonadal hormones on the nucleic acid content of liver and serum in the immature pullet, and the difference between the nucleic acid content of the livers of sexually mature pullets and cockerels. Can. J. Zool. 29 : 265–275. 1951.
4. COMMON, R. H., KEEFE, T. J., and MAW, W. A. Some biochemical effects of thiouracil on the response of the immature pullet to estrogen. Can. J. Research, D, 28 : 272–279. 1950.
5. COMMON, R. H., RUTLEDGE, W. A., and BOLTON, W. The influence of gonadal hormones on serum riboflavin and certain other properties of blood and tissues in the domestic fowl. J. Endocrinol. 5 : 121–130. 1947.
6. FLEISCHMANN, W. and FRIED, I. A. Studies on the hypercholesterolemia and hypercalcemia induced by estrogen in immature chicks. Endocrinology, 36 : 406–415. 1945.
7. FRAPS, R. M. and DURY, A. Occurrence of premature ovulation in the domestic fowl following the administration of progesterone. Proc. Soc. Exptl. Biol. Med. 52 : 346–349. 1943.
8. FRAPS, R. M., HERTZ, R., and SEBRELL, W. H. Relation between ovarian function and avidin content in the oviduct of the hen. Proc. Soc. Exptl. Biol. Med. 52 : 140–142. 1943.
9. FRAPS, R. M., HOOKER, C. W., and FORBES, T. R. Progesterone in blood plasma of the ovulating hen. Science, 108 : 86–87. 1948.
10. HERTZ, R., FRAPS, R. M., and SEBRELL, W. H. Induction of avidin formation in the avian oviduct by stilbestrol plus progesterone. Proc. Soc. Exptl. Biol. Med. 52 : 142–144. 1943.
11. HERTZ, R., LARSEN, C. D., and TULLNER, W. Inhibition of estrogen-induced tissue growth by progesterone. J. Natl. Cancer Inst. 8 : 123–126. 1947.
12. NEHER, B. H. and FRAPS, R. M. The addition of eggs to the hen's clutch by repeated injections of ovulation-inducing hormones. Endocrinology, 46 : 482–488. 1950.
13. ROTHCHILD, I. and FRAPS, R. M. The interval between normal release of ovulating hormone and ovulation in the domestic hen. Endocrinology, 44 : 134–140. 1949.
14. SCHNEIDER, W. C. Phosphorus compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid and of pentose nucleic acid. J. Biol. Chem. 161 : 293–303. 1945.
15. SELYE, H. Interactions between various steroid hormones. Can. Med. Assoc. J. 42 : 113. 1940.

THE AEROBIC BACTERIA ASSOCIATED WITH THE EASTERN HEMLOCK LOOPER *LAMBDINA FISCELLARIA* (GN.)[1]

By T. A. Angus[2]

Abstract

A study was made of healthy hemlock loopers (*Lambdina fiscellaria* Gn.) to determine whether a specific bacterial flora is associated with this insect. Larvae, pupae, adults, and eggs were examined by a number of methods and the bacteria found were cultured and classified. Organisms belonging to nine genera of bacteria were isolated and they were principally of foliage-contaminating or soil types that could be ingested with the insects' food. Few of the ingested bacteria survive the digestive process. It is concluded that the bacterial flora is adventitious.

Introduction

The published work on the bacterial flora of insects has been reviewed by Steinhaus (7). Heimpel (5) studied the normal flora of the tent caterpillar, *Malacosoma americanum* (F.), and found it to be adventitious. Thomson (8) reported that *Pseudomonas* spp. were associated with the larval gut of the spruce budworm, *Choristoneura fumiferana* (Clem.). The purpose of the present study was to determine whether a specific aerobic bacterial flora is associated with the eastern hemlock looper, *Lambdina fiscellaria* Gn.

Materials and Methods

Hemlock looper larvae were obtained from a number of locations in Ontario, New Brunswick, and Newfoundland. The insects were reared in large cages in an outdoor insectary and fed fresh balsam fir foliage obtained from sites well removed from roads and paths. No attempt was made to cleanse the foliage but precautions were taken to reduce the risk of casual contamination during handling. Insects in various stages of development were examined by one of the methods given below.

A number of compounds have been used for the surface cleansing of insects and some of these have been discussed by Steinhaus (7). In this study, a quaternary ammonium salt, Hyamine 1622 (isobutyl-phenoxy-ethoxy-ethyl-benzyl-ammonium chloride), was used since its properties make it a very useful agent for this purpose. It is surface active, easily soluble in water, and, in dilute solutions, nontoxic to many insects. Insects were cleansed by immersing them, with frequent agitation, in a 0.2% aqueous solution of Hyamine 1622. Immersion was followed by two rinsings in sterile water. In some instances a 0.1% solution was used and this is indicated in the description of the method used.

All cultures were incubated at 25° C.

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Contribution No. 50, Division of Forest Biology, Science Service, Department of Agriculture, Ottawa, Canada. This paper is a summary of a thesis presented to the University of Toronto in partial fulfillment of the requirements for the degree of Master of Science (Agriculture).

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(*a*) Living larvae with the oral and anal openings plugged with wax, and pupae and adults with appendages removed, were anaesthetized with chloroform and washed. The cleansed insect was transferred to a tube of nutrient broth (Difco) and incubated for 48 hr. If growth was not observed in this time, it was assumed that surface sterilization had been achieved and the insect was then triturated with a glass rod. The resultant suspension was incubated.

(*b*) Blood samples were collected from cleansed larvae. Two shallow incisions, dorsal anterior and dorsal posterior, were made into the body cavity with dissecting scissors. A platinum loop was used to collect a drop of blood which was inoculated into a tube of nutrient broth and incubated. Stained films were made of several blood samples.

(*c*) The alimentary tracts of cleansed larvae were removed and examined. The head and the last abdominal segment were cut off and the intestinal tract removed with forceps. The tubelike gut was ligatured with silk thread and immersed for three minutes in a 0.1% Hyamine solution. After a rinse in sterile water, the ligatured gut was transferred to a tube of nutrient broth and incubated for 48 hr. If growth was not observed in this time, the gut was triturated and the suspension incubated. The ligatured gut was composed of the entire mid-gut but only part of the fore- and hind-guts.

(*d*) Frass samples were collected from living larvae which had been anaesthetized and immersed for five seconds in a 0.1% Hyamine solution. The wet insects were dried on sheets of sterile bibulous paper and placed in individual tubes on a strip of filter paper. Eventually the insects revived and defecation occurred. After several frass pellets had accumulated, the insect was removed by lifting out the paper strip. A quantity of broth was added to the frass pellets and incubated. Stained films were made of several frass pellets.

(*e*) Various organs, such as the alimentary tract, ovaries, or testes, were removed from cleansed adult insects. The dissections were carried out as rapidly and aseptically as possible in sterile saline, and the organs were placed in tubes of nutrient broth, triturated, and incubated. Additional information about the female reproductive organs was provided by inspection of eggs.

(*f*) Female moths were anaesthetized and immersed for five seconds in a 0.1% Hyamine solution. The wet insects were transferred individually to large sterile jars containing crumpled bibulous paper which acted as a drying agent and provided a surface on which eggs could be laid. After 20 to 30 eggs had been laid, the insect was removed and the eggs transferred to tubes of nutrient broth and incubated. If this test showed absence of external microorganisms, the eggs were triturated and incubated to determine whether any bacteria were present internally. These eggs had not been surface cleansed so there was no possibility of adsorption of Hyamine.

(*g*) Eggs laid in rearing cages were collected and examined for the presence of internal microorganisms. The eggs were divided into lots of about 50 and each lot was washed for 10 min. in a 0.2% Hyamine solution. The washed

eggs were placed in a sterile filter paper cone in a covered funnel and rinsed several times with sterile water, transferred to tubes of nutrient broth, triturated, and incubated.

The broth suspensions of organs, blood samples, frass pellets, eggs, and whole insects were streaked on poured plates of blood agar base (Difco) medium. Discrete homogenous colonies on these plates were transferred to agar slants, incubated for 48 hr., and stored at 6° C. These constituted the stock cultures of the microorganisms. The isolated organisms were studied using accepted procedures and keyed out to genera.

Results and Conclusions

The results of the various examinations are summarized in Table I. In addition to bacteria and yeasts, a number of fungi were isolated from the larval gut and frass but no attempt was made to classify them. Casual inspection revealed that *Penicillium* and *Aspergillus* species predominated.

TABLE I

TYPES OF BACTERIA ISOLATED FROM THE HEMLOCK LOOPER

	Number of cultures isolated from						
	Larvae				Pupae	Adult	
	Whole	Blood	Gut	Frass	Whole	Whole	Organs
Aerobacter sp.	1				4		
Achromobacter sp.	2		3		1	2	
Bacillus sp.	12	2	6		3	5	3
Bacterium sp.	3				1	1	
Escherichia sp.	1						
Flavobacterium sp.	1				2		
Micrococcus sp.	5	1	2	1	1	1	1
Paracolobactrum sp.	1					1	
Proteus sp.			1			1	
Number of cultures	26	3	12	1	12	11	4
Number of tests made	50	70	70	45	68	43	35
Number of yeast cultures	9	1	17	16	11	2	1

NOTE: *The exterior surface of 250 eggs laid in a sterile environment proved to be sterile; 500 eggs collected from rearing cages were triturated and proved to be sterile internally.*

All the yeasts isolated were very similar and appeared to be species of the genus *Cryptococcus*. Yeasts were the most commonly occurring microorganisms in the frass.

Bacteria were isolated from crushed whole larvae and from the larval alimentary tract, but larval frass was usually free of bacteria, which suggests that ingested bacteria do not survive or that their numbers are greatly reduced in the gut. The more frequent isolation of bacteria from crushed whole

larvae than from ligatured guts can be explained by assuming that bacteria ingested with the food are viable while in the fore-gut, but that they are less viable in the mid-gut. Staudenmeyer (6) and Waterhouse (9) reported that mid-gut alkalinity is characteristic of the order Lepidoptera and limited tests showed this to be true for the hemlock looper. Such alkalinity may be unfavorable for the survival of ingested bacteria. In support of this, it was noted that bacteria were isolated more frequently from guts distended with recently ingested food than from guts where the digestive cycle was more advanced.

No microorganisms were seen in the stained blood films and practically all the blood cultural tests were negative. This is at variance with the findings of Cameron (2) who found bacteria in the blood of normal caterpillars of several species.

Only a preliminary examination was made to determine whether anaerobic bacteria are associated with the hemlock looper. Thirty larvae were cleansed, triturated in tubes of nutrient broth, and incubated under conditions of reduced oxygen tension. No anaerobic species were isolated with the methods used.

One of the most serious shortcomings of this study is that a general purpose medium was used. Steinhaus (7) recommended nutrient blood or chocolate blood agar, North's gelatin chocolate blood agar, and glucose agar. Initially these were used but they proved to be no more effective than the sequence adopted. Possibly there are fastidious bacteria associated with the hemlock looper which were not isolated by the procedures used. This problem has been discussed by Gier (3) and Gubler (4) who tried a great many media in their attempts to culture intracellular symbionts of insects.

If there were a specific microflora associated with the hemlock looper, we would expect to isolate the bacterial species composing it from all or from a majority of the insects examined. Organisms of the genus *Bacillus* occurred frequently, but a comparison of the biochemical characteristics of the isolates showed that at least six different species were present (Table I). The same was true of the *Micrococcus* types isolated. A complete description of each of the cultures isolated from the hemlock looper is given elsewhere (1).

It is concluded that there is no specific aerobic bacterial flora associated with the eastern hemlock looper. The eggs are laid in a sterile condition and no bacterial species is transmitted by way of the egg. Bacteria can be isolated from the gut but these appear to be organisms ingested with the food. The number of bacteria decreases as the food is digested and the frass pellets do not usually contain viable bacteria. Certain yeasts and mold spores pass through the gut and can be cultured from the frass pellets.

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References

1. ANGUS, T. A. The normal aerobic bacterial flora of the eastern hemlock looper. M.S.A. thesis, University of Toronto. 1950.
2. CAMERON, G. R. Inflammation in caterpillars of lepidoptera. J. Path. Bact. 38 : 441–446. 1934.
3. GIER, H. T. Intracellular bacteroids in the cockroach. J. Bact. 53 : 179–189. 1947.
4. GUBLER, H. V. Attempts to culture intracellular symbionts of insects. Schweiz. Z. Path. u. Bakt. 11 : 489–493. 1949. *Cited in* Biol. Abstracts 11252. 1949.
5. HEIMPEL, A. M. The normal bacterial flora and pathogens of the eastern tent caterpillar. M.Sc. thesis, Queen's University. 1949.
6. STAUDENMEYER, TH. Die Wasserstoffionenkonzentration der Insekten. Anz. Schädlingskunde, 16 : 114–132. 1940.
7. STEINHAUS, E. A. Insect microbiology. McGraw-Hill Book Company, Inc., New York. 1946.
8. THOMSON, H. H. Aerobic bacteria associated with the digestive tract of the spruce budworm. M.Sc. thesis, McGill University. 1951.
9. WATERHOUSE, D. F. The hydrogen ion concentration in the alimentary canal of larval and adult lepidoptera. Australian J. Sci. Research, B, 2 : 428–437. 1949.

FACTORS INFLUENCING THE PERFORMANCE OF THE PRECIPITIN TEST IN THE DETERMINATION OF BLOOD MEALS OF INSECTS[1]

By G. S. Eligh[2]

Abstract

Certain factors influence the performance of precipitin tests when the latter are used for detection of blood meals of biting flies. Antisera stored in a liquid state in a refrigerator generally show a decrease in titer with the passage of time. Aging appears to have an adverse effect on the avidity of an antiserum. Smears of blood-engorged mosquitoes prepared by smearing of the entire insect are satisfactory for testing by the precipitin reaction. A high-titered antiserum which can be diluted is desirable for detection of host blood in mosquitoes.

Introduction

The precipitin test is widely used in identifying the host blood of biting flies (7, 8, 10). The test is also used in taxonomic studies (5) and one paper (2) reports on the use of the test to identify the hosts of predatory insects.

The serological principles of the precipitin test are well established, but there have been few detailed studies on the application of this technique to entomological problems. At Queen's University, Kingston, the precipitin technique is being developed as an entomological tool (11). The identification of blood meals of biting flies of the Canadian north and of hosts of various predatory insects are being investigated at present.

Initial studies showed that the precipitin test could not be treated as a routine laboratory procedure. In the literature a number of workers briefly mention various factors that influence the performance of precipitin tests. This paper describes the procedures used at Kingston. Detailed studies on the effects of aging on the titers of antisera, on the deterioration of avidity of fresh antisera, and on the methods of preparation of smears are reported.

Methods

Rabbits were used for production of antisera. Intravenous injections of 0.5 ml., 1 ml., and 1.5 ml. of serum were given on alternate days. Trial bleedings were taken 12–15 days after the final injection. If the titer was found to be suitable, 1 : 6400 or more as recommended by Arnold and coworkers (1), the rabbit was bled aseptically from the marginal vein of the ear. Separation of the immune serum from the blood clot was facilitated by centrifuging. The antiserum was placed in sterile, rubber-stoppered vials and

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merthiolate (freshly prepared 1 : 500 stock solution) was added as a preservative up to a concentration of 1 : 10,000. The vials of preserved antiserum were stored in a cold room at approximately 5° C.

All precipitin tests were done by the "ring test" method. To find the titer of an antiserum the procedure was as follows. Serial dilutions of antigen (serum) were made beginning with a dilution of 1 : 100 and ending with 1 : 50,000. Buffered saline (3) was used to make the dilutions. About 0.1 cc. of each antigen dilution was transferred to a test tube 5 mm. in diameter by 40 mm. long. Undiluted homologous antiserum was layered under the antigen using a finely drawn pipette made from 3 mm. bore tubing. After incubation for half an hour at room temperature, the reactions were read under an incandescent lamp before a black background. The highest dilution of antigen that showed a perceptible ring of precipitate was recorded as the titer.

When the titer had been established progressive dilutions of antiserum were made: 1:3, 1:6, 1:12, etc., with 50% glycerol–saline. Each of these dilutions was layered under the serial dilutions of antigen as before. The antiserum was then used at the highest dilution which produced easily read reactions over the entire range of antigen dilutions bounded by the titer. The ability of the antiserum to react when diluted is a measure of its avidity. For practical purposes when extracts of insect smears are tested with an antiserum, the important consideration is the ability of that antiserum to detect high dilutions of the homologous antigen in a short time rather than a study of the reaction of the antiserum by optimal proportion methods.

Smears of blood-engorged mosquitoes (methods of smear preparation are discussed later in this paper) were made on a high quality filter paper (Stanscien 333). The filter paper was cut as close to the edge of the smear as possible. This small disk was placed in a 5-ml. beaker and 1.5 ml. of buffered saline was added and allowed to stand approximately one and one-half hours at room temperature. About 0.1 ml. of the saline extract was transferred by pipette into a small test tube. The antiserum, in appropriate dilution, was layered under this extract. To aid in detection of false negatives or positives, a negative control (antiserum layered under saline) and a positive control (antiserum layered under 1 : 1000 homologous antigen) was set up. Readings were taken as already described after incubation for half an hour at room temperature.

Effect of Aging on the Titer of an Antiserum

When experimental procedures and circumstances are such that considerable time elapses before prepared antisera are used it becomes important to know whether a drop in titer occurs. Brooke and Proske (2) stated that the titer of most antisera diminishes gradually in storage except when the sera are dehydrated from the frozen state. They reported an instance in which one antiserum that had a titer of 1 : 4320 originally and that was used for experimental tests during "several weeks" was then found to have a titer between 1 : 480 and 1 : 960.

During 1949 a number of antisera were prepared, titered, and stored under the conditions outlined above. Approximately one year later these antisera were again titered. It was established that the antisera were free of contaminants. The original titer, the age in months, and the titer after aging are recorded for 13 antisera in Table I. With the exception of antirabbit and anti-

TABLE I

THE EFFECT OF AGING ON THE TITERS OF ANTISERA

Antiserum	Age, months	Original titer	Titer after aging
Rabbit*	14	1 : 8000	1 : 8000
Porcupine	13	1 : 32000	1 : 1000
Cow	14	1 : 8000	1 : 4000
Human	13	1 : 32000	1 : 500
Barren ground caribou	14	1 : 16000	1 : 4000
Swine	14	1 : 8000	1 : 1000
Buffalo	13	1 : 8000	1 : 8000
Painted turtle	13	1 : 32000	1 : 500
Dog	14	1 : 16000	1 : 8000
Raccoon	13	1 : 32000	1 : 4000
Elk	13	1 : 8000	1 : 2000
Pigeon	12	1 : 8000	1 : 4000
Chicken	13	1 : 32000	1 : 4000

* *Rabbit antiserum was prepared in chicken. All other antisera were prepared in rabbits.*

buffalo sera, in which there was no change, all the antisera showed a drop in titer. As will be observed in the table the antiserum having the highest titer showed the greatest loss. The presence of a fine precipitate was noted in many of these old antisera. This precipitate may be associated with protein denaturation. From these data it is apparent that the titer of an antiserum generally decreases with age. This phenomenon has been commonly observed. The extent of decrease in titer as shown in Table I is not necessarily representative.

Deterioration of the Avidity of Fresh Antisera

Deterioration of avidity was noted in comparatively fresh antisera. A rabbit which had been immunized with human serum and had responded satisfactorily was bled twice with an interval of 12 days between bleedings. Each lot of antiserum had a titer of 1 : 12,500 and could be diluted to 1 : 96 with 50% glycerol–saline and still produce a visible reaction over the entire range of antigen dilutions bounded by the titer. The first lot of antiserum (1 bottle of 20 ml.) was tapped for routine tests at irregular intervals over a period of four months. Enough antiserum was withdrawn from the bottle by a sterile syringe to complete the particular test. The bottle of antiserum was subjected to room temperature for approximately 15 min. before being returned to the cold room. The antiserum was used in this way until it was noted that it no longer gave strong positive reactions with human blood

extracted from mosquito smears. No bacterial contamination was present. The second lot of antiserum had been stored untouched in the cold room. Neither antiserum showed any visible evidence of deterioration. The titers of both lots of antiserum were determined again. Progressive dilutions of each antiserum were made and the maximum dilution of antigen detected by each dilution of antiserum (i.e., the titer) was recorded. The data are presented in Table II.

TABLE II

THE TITERS OF COMPARATIVELY FRESH ANTIHUMAN SERA WHEN TAPPED FREQUENTLY AND WHEN STORED WITHOUT TAPPING

Dilution of antiserum	Antihuman serum 132 days old tapped frequently		Antihuman serum 120 days old not tapped	
	Titer	Reading*** time, min.	Titer	Reading time, min.
Undiluted	1 : 12,500	30	1 : 12,500	15
1 : 3	1 : 12,500	30	1 : 12,500	15
1 : 6	No reaction	60	1 : 25,000*	15
1 : 12	No reaction	60	1 : 25,000	15
1 : 24			1 : 25,000	15
1 : 48			1 : 25,000	15
1 : 96			1 : 12,500**	15
1 : 192			No reaction	60

* *This apparent increase in titer is probably due to removal of antibody excess through dilution.*

** *The titer at 30 min. was 1 : 25,000.*

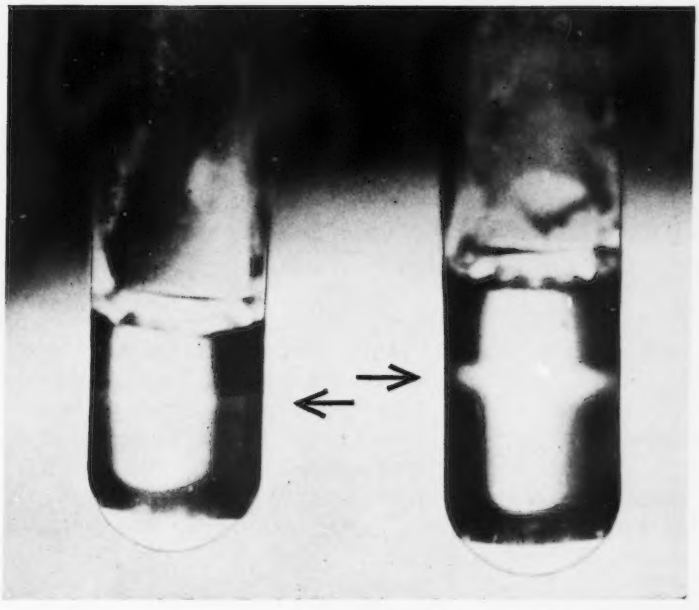
*** *Observations were made every 15 min.*

Though these antisera had the same titer there was a great difference in avidity. The first lot of antiserum which had been tapped frequently gave lighter precipitates and required a longer time to react. It would not react when diluted even moderately. The second lot of antiserum produced light but easily read reactions at the high dilution of 1 : 96. With lesser dilutions the reactions were proportionately heavier.

A test was done in which both these antisera were diluted 1 : 3 with 50% glycerol–saline and layered under 1 : 1000 dilutions of human serum. The first antiserum showed a precipitate after 20 min. whereas the second showed a heavy precipitate after eight minutes (Fig. 1).

The evidence presented suggests that frequent tapping might have some effect concerned with deterioration of an antiserum. To avoid this possibility all antisera on hand were transferred to 4 ml. vials. Tables I and II suggest that titer and avidity are separate entities. Wolfe and Baier (12) stated: "Indications are that at least two types of antibodies are present: (1) a 'titer' producing antibody and (2) a precipitate forming antibody." They reported an experiment in which antisera produced different amounts of precipitate and suggested that age might be a contributing factor. It is worthy of mention

PLATE I



A B

FIG. 1. A comparison of the degree of reaction when two antisera of the same titer and different avidity are used. *A*. Nonavid antiserum gives light precipitate after 20 min. *B*. Avid antiserum gives heavy precipitate after 8 min.

that fresh antisera may have a very high titer and at the same time be comparatively nonavid.

An additional experiment was performed to emphasize the value of a high-titered and avid antiserum for study of insect blood meals. Two antisera were used: relatively fresh antiserum of comparatively low avidity and low titer (1 : 2500) and the avid, high-titered (1:12,500) antiserum used in the study reported in Table II. Both antisera were set up against extracts of 10 smears of mosquitoes (*Aedes aegypti* (L.)) that had been engorged with human blood and held for eight days at 11° C. The antiserum of low avidity, diluted 1:5, gave positive tests with five of the 10 smear extracts. The avid antiserum, diluted 1 : 24, gave positive tests with all 10 smear extracts. These results show that an avid antiserum of high titer is most suitable for use in detection of blood that may be partially digested by the mosquito before it is smeared.

Preparation of Mosquito Smears

Smears of blood-engorged insects have been prepared in a variety of ways for subsequent testing by the precipitin reaction. Lloyd, Napier, and Smith (6) did precipitin tests on blood from engorged sand flies. They dissected the living flies immediately after capturing them. The engorged gut was removed and placed in the tube in which the reaction was to be carried out. Johnson and Rawson (4), working with tsetse flies, dissected the flies immediately after capture and pressed the whole blood out of the gut onto filter paper, where it was allowed to dry. Riddell *et al.* (9) prepared smears by severing the abdomen of the mosquito and expressing the contents of the abdomen on filter paper, where it was allowed to dry.

Preparation of specimens by dissection is a laborious and time-consuming process. Severing of the abdomen and expressing its contents also requires concerted attention and careful manipulation. If specimens could be prepared merely by vigorous smearing of the entire insect, the preparation of insect smears in the field would be greatly facilitated. To determine the most suitable method for preparation of mosquito smears, a number of *Aedes aegypti* (L.) engorged with human blood were prepared in each of two ways. One lot consisted of mosquitoes smeared on the filter paper by heavy pressure of thumb and forefinger wiping the member clean on the paper. A second lot was prepared by teasing away the tip of the abdomen and expressing the contents on the filter paper. This method is somewhat comparable to that of Riddell *et al.* (9). The smears of both lots were allowed to dry thoroughly and were stored until used. When the smears were extracted, specimens prepared by vigorous smearing of the entire insect gave a clear extract, whereas those prepared by expressing the abdominal contents almost invariably gave a cloudy extract. When these extracts were set up against avid antihuman serum, the clear extract always gave a sharp positive test that could be read easily. The cloudy extracts did not give such readily discernible reactions and in certain cases had to be read as doubtful.

Rice and Barber (8) stated that blood which had permeated the fibers of the filter paper did not yield its serum readily when extracted with saline. This observation was tested by smearing a number of mosquitoes immediately after they had engorged themselves with human blood. The fresh blood was pressed from the mosquito and allowed to soak into the filter paper and dry. When these smears were extracted with saline for about one and one-half hours at room temperature and the extract was set up against an antihuman serum of 1 : 2500 titer, there were no positive reactions. This same antiserum gave positive results when tested against mosquito smears made by vigorous smearing of the entire insect. Possibly when the entire insect is smeared some of the pores of the paper are clogged by insect body material and the blood dries more or less as a clot, which is more easily extracted. If the engorged mosquitoes are allowed to remain alive for some time, the blood appears to thicken in the gut. When such mosquitoes are smeared this thick blood does not readily soak into the paper. Extracts of such smears give excellent results when used for precipitin tests.

Acknowledgments

The writer wishes to thank Dr. A. S. West, Department of Biology, Queen's University, for his direction of the work and for his help in the preparation of this paper.

References

1. ARNOLD, E. H., SIMMONS, S. W., and FAWCETT, D. G. Precipitin technique for determining mosquito blood meals. U.S. Pub. Health Service, Pub. Health Repts. 61 : 1244–1249. 1946.
2. BROOKE, M. M. and PROSKE, H. O. Precipitin test for determining natural insect predators of immature mosquitoes. J. Natl. Malaria Soc. 5 : 45–56. 1945.
3. EVANS, A. C. A buffered physiologic salt solution. J. Infectious Diseases, 30 : 95–98. 1922.
4. JOHNSON, W. B. and RAWSON, P. H. Use of the precipitin test to determine the food supply of tsetse flies; a preliminary note. Trans. Roy. Soc. Trop. Med. Hyg. 21 : 135–149. 1927.
5. LEONE, C. A. A serological study of some Orthoptera. Ann. Entomol. Soc. Am. 40 : 417–433. 1947.
6. LLOYD, R. B., NAPIER, L. E., and SMITH, R. O. A. The blood meal of *Phlebotomus argentipes* identified by precipitin antisera. Indian J. Med. Research, 12 : 811–817. 1924.
7. REMPEL, J. G., RIDDELL, W. A., and MCNELLY, E. M. Multiple feeding habits of Saskatchewan mosquitoes. Can. J. Research, E, 24 : 71–78. 1946.
8. RICE, J. B. and BARBER, M. A. Malaria studies in Greece. A modification of the Uhlenhuth-Weidanz precipitin test for determining the sources of blood meals in mosquitoes and other insects. J. Lab. Clin. Med. 20 : 876–883. 1935.
9. RIDDELL, W. A., REMPEL, J. G., and MCNELLY, E. M. The specificity of the precipitin reaction as used in the study of mosquito feeding habits. Can. J. Research, E, 25 : 210–215. 1947.
10. SCHUBERT, J. H. and KELLEY, M. H. The precipitin technique for determining species of host blood in mosquitoes—modifications and improvements. J. Natl. Malaria Soc. 9 : 341–348. 1950.
11. WEST, A. S. The precipitin test as an entomological tool. Can. Entomologist, 82 : 241–244. 1950.
12. WOLFE, H. R. and BAIER, J. G. Comparison of quantitative precipitin techniques as influenced by injection procedures. Physiol. Zool. 11 : 63–74. 1938.

THE BOTTOM SEDIMENTS OF LAKE LAUZON, MONTCALM COUNTY, PROVINCE OF QUEBEC

I. CHEMICAL AND PHYSICAL INVESTIGATIONS[1]

By Herman Kleerekoper[2] and F. Grenier[3]

Abstract

Macroscopic, microscopic, and chemical examinations of 287 bottom samples collected in Lake Lauzon, Montcalm County, P.Q. by means of core sampler, combination sounding weight, and Ekman dredge showed that most of the bottom of the lake is covered with dark brown to blackish sludge whose thickness varies from 3.5 to more than 350 mm. It is of semiliquid nature but becomes colloidal in well defined areas. The suspensions contain hydrogen sulphide. The water content varied between 31 and 95%. The highest loss on ignition observed was 75.0%, in the deeper parts of the lake. The ratio $\frac{\text{lignin}}{\text{loss on ignition}}$ varies between 15.0 and 64.7. It is estimated that lignin accounts for half of the organic matter of the surface bottom deposits. Nitrogen varies between 0.00 and 1.68%. Samples with high loss on ignition contained also the higher amounts of nitrogen. The ratio $\frac{\text{nitrogen}}{\text{loss on ignition}}$ varies between 0.00 and 3.09. There was no correlation between the location of the sample in the lake and this ratio. Silica content is closely related to depth and to position in relation to the shore. In the deepest part (24.4 m.), 32.7% was found. One sample contained 82.4% silica. Calcium varied between 0.34 and 1.69% in the surface sediments. There is no correlation between depth and calcium content. The ratio Ca/Mg varies between 1.55 and 29.0. In half of the samples it was between 2 and 4. Samples with a higher ratio (above 20) contained slightly more nitrogen than the average for all samples (0.04). Phosphorus varied between traces and 0.42%. Samples with higher loss on ignition contained generally the highest amount of phosphorus, although some exceptions were observed. A large part of the phosphorus in the surface sediments seemed to be in organic form. There was no apparent correlation between iron and phosphorus contents. About one-third of the samples contained more than 1% iron. Values for this element varied between 0.19 and 3.87%. The average chemical composition in % dry weight of all surface bottom sediments was: loss on ignition 45.6; crude lignin 17.08; ashes 54.4; total N 0.94; SiO_2 46.0; Al_2O_3 7.11; Ca 0.87; Mg 0.21; total P 0.14; total Fe 1.02; percentage of water 84.9. For a chemical and microscopical study of the subsurface strata, 128 cores were taken. The bacteriology of these samples was described elsewhere. The surface layer of sludge is underlain by different layers which were classified as amorphous organic matter, nonfibrous plant detritus, fibrous plant detritus, sandy organic detritus, sandy clay, fine sand, and coarse sand. A profile of the lake bottom was prepared to a depth of about 40 cm. Chemical analyses were carried on for the surface samples. A study was made of the relative abundance in all samples of diatoms, sponge spicules, pollen grains, wood fibers, remains of mosses, plankton, and tubes of worms.

Introduction

The present study is part of an extensive limnological investigation started in June 1949 by the staff of the Biological Station of Mont Tremblant, and was carried out simultaneously with general biological and bacteriological studies on the lake sediments as well as with intensive investigations on several other limnological factors in Lake Lauzon. The results of a number of these

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investigations are being published as *Contributions of the Biological Station of Mont Tremblant.* The chemical and physical investigations were closely correlated with the bacteriological study made on the same sediments by Cooper, Murray, and Kleerekoper (4). Similar studies on the sediments of other lakes in the Mont Tremblant Park area are now in progress. In this way an attempt will be made to characterize the lakes of the area from a regional limnological point of view, and to correlate these characteristics with present conditions of biological productivity in these lakes. It will also be attempted to use the data on the deep sediments for an understanding of the biological conditions which were likely to have existed in the past. In planning these investigations, special attention was given to those chemical elements which are likely to represent limiting factors in the biological productivity of the lakes of this region. It was hoped that by correlating chemical, physical, and bacteriological observations a better understanding would be obtained of the processes of mineralization of organic compounds in the lake, and of the cycles of the important nutrients on which optimal development of phytoplankton depends.

Owing to the relative absence of silting, of large fluctuations in the water level, of disturbances produced by high winds, and of permanent inlets and outlets, Lake Lauzon (Figs. 1 and 2) was selected for the detailed study of certain ecological problems of fundamental significance related to lake productivity. Although the chemical and biological characteristics of the bottom sediments of other lakes are also being studied, special attention has been and will further be given to those of Lake Lauzon.

Certain Limnological Characteristics of Lake Lauzon

Lake Lauzon is located at 74°, 30′, 45″ W. and 46°, 21′, 0″, N. in Mont Tremblant Park, Montcalm County, Province of Quebec, at 2 miles from the Biological Station of Mont Tremblant (Fig. 1). A map of the bottom contours of the lake was made in 1949 based on measurements carried out by means of a transit (Fig. 2). The hydrographical characteristics of the lake are shown in Table I.

TABLE I

LAKE LAUZON. HYDROGRAPHIC DATA

Surface area	106,000 m.² (26 ac.)
Total volume	1,085,000 m.³ (1,413,000 cu. yd.)
Mean depth	10.2 m. (33.4 ft.)
Maximum depth	24.4 m. (86.6 ft.)
Volume of epilimnion on July 20, 1950	319,000 m.³
Volume of hypolimnion on " " "	399,000 m.³
Volume of metalimnion on " " "	362,000 m.³

There are no permanent outlets or inlets and the fluctuations of the level of the lake water are small (about 40 cm.). The lake is completely surrounded by mixed forest and hills, of which the highest is 2000 ft. (610 m.), and is supplied almost exclusively by ground water and seepage. The maximum

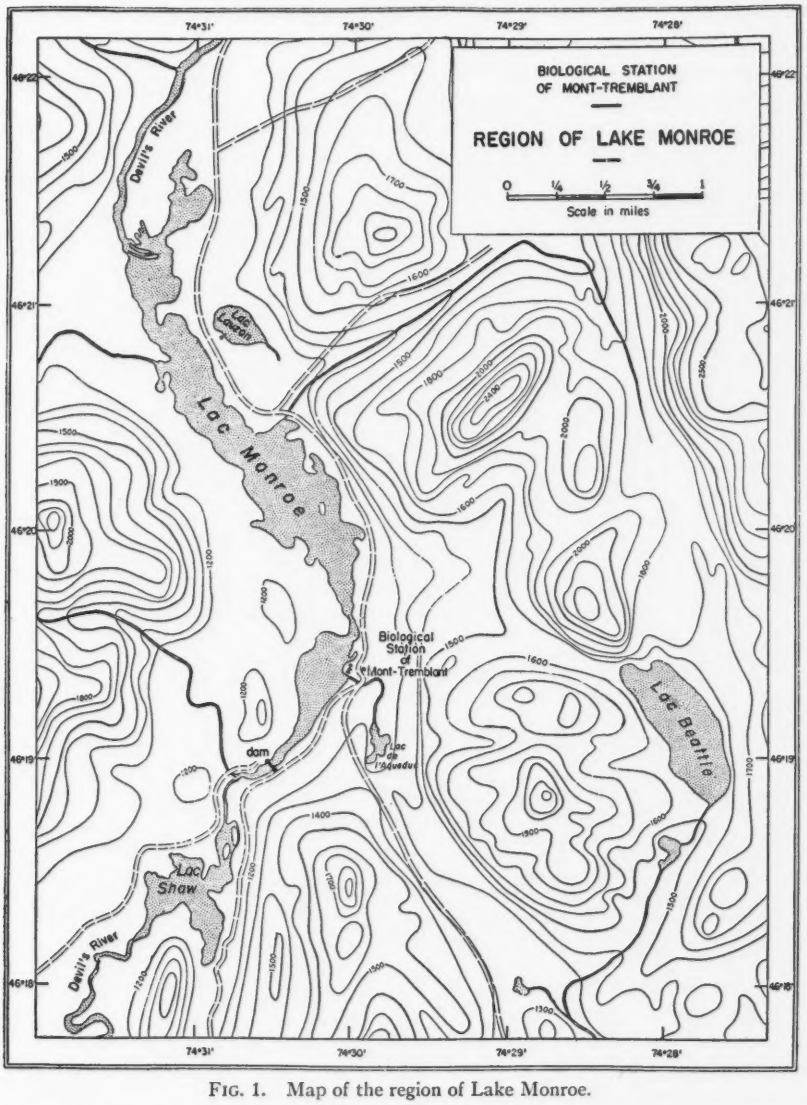

FIG. 1. Map of the region of Lake Monroe.

level of the water was observed in June 1950 during a period of heavy rainfall when excess water ran off into Lake Monroe through the temporary outlet at the southeastern end of the lake. The drainage area of the lake is small (about 320 ac. or 129 ha.), and is completely covered by vegetation which advances up to the edge of the water. The shore line is formed mainly by thick deposits of conifer leaves. The absence of a permanent inlet and the

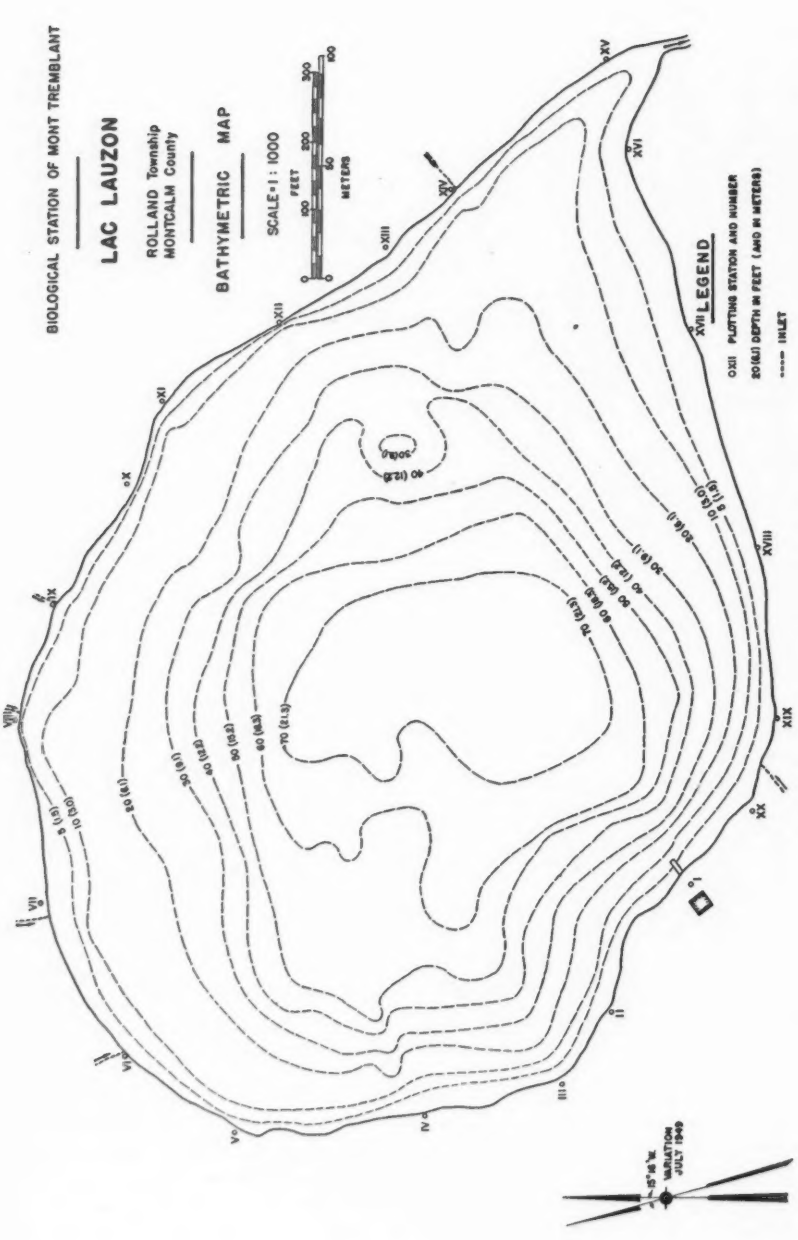

FIG. 2. Bathymetric map of Lake Lauzon.

dense vegetation of the drainage area practically exclude silting materials from the lake in its present condition. The loose deposits of conifer leaves which reach, at some places, a depth of at least 8 ft. (2.4 m.) further contribute to protect the lake from the inflow of materials carried by whatever runoff might occur. The lake is also well protected from high winds by the forest –covered hills by which it is surrounded. This condition is well shown in its thermal characteristics which were described by Kleerekoper (10). The wind-distributed heat on Sept. 7, 1949, was calculated at 11,372 gm-cal. (10).

The lake is of the extreme oligotrophic type with very low plankton crops throughout the summer months. Table II presents some chemical data on the water of the lake (12).

TABLE II

LAKE LAUZON. CERTAIN CHEMICAL CHARACTERISTICS OF THE WATER OBSERVED AT STATION 36 DURING THE SUMMER OF 1949

Date, 1949	Number of sample	Depth in meters	T, ° C.	pH	CO_2, p.p.m.	O_2, p.p.m.	Bicarbonate, p.p.m.
July 27	A- 1	0.0	23.5	6.8	2.4	7.59	4.7
	A- 2	—	—	—	—	—	—
	A- 3	1.0	23.0	6.8	1.8	7.49	4.7
	A- 5	2.0	22.6	6.7	1.2	7.48	4.7
	A- 7	4.0	22.0	6.7	1.2	7.03	4.7
	A- 9	22.0	7.0	—	19.2	—	—
	A-10	23.0	5.0	6.1	15.6	0.86	9.1
Aug. 1	A-12	0.0	24.5	—	0.6	7.01	—
	A-13	4.0	23.5	—	0.6	6.94	—
	A-14	5.0	21.6	—	0.6	8.19	—
	A-15	6.0	18.5	—	1.2	9.82	—
	A-16	7.0	14.5	—	1.7	9.82	—
	A-17	10.0	8.5	—	1.7	9.35	—
	A-18	11.0	8.0	—	1.7	9.54	—
Aug. 4	A-24	0.0	24.0	—	0.6	7.13	—
	A-25	4.0	23.0	—	0.6	7.07	—
	A-26	5.0	22.5	—	0.6	7.4	—
	A-27	6.0	17.5	—	1.2	9.85	—
	A-28	7.0	—	—	1.2	9.6	—
	A-29	8.0	11.5	—	1.2	9.12	—
	A-30	9.0	10.0	—	1.2	9.12	—
	A-31	10.0	8.5	—	1.7	9.22	—
	A-32	11.0	7.0	—	2.3	9.06	—
	A-33	14.0	5.5	—	2.9	7.75	—
	A-34	21.0	5.2	—	4.1	3.14	—
Aug. 8	A-35	0.0	24.5	6.6	0.6	9.02	—
	A-36	4.0	23.5	6.8	0.6	6.78	—
	A-37	5.0	23.0	6.6	0.6	7.19	—
	A-38	6.0	18.5	6.8	1.1	9.66	—
	A-39	7.0	13.5	6.8	—	—	—
	A-40	8.0	11.0	6.6	1.1	9.74	—
	A-41	9.0	9.5	6.5	1.7	9.04	—
	A-42	10.0	8.5	6.6	1.7	9.66	—
	A-43	11.0	7.5	—	1.7	9.45	—
	A-44	12.0	7.5	6.4	2.2	9.12	—
	A-45	13.0	7.5	6.3	2.2	8.51	—
	A-46	17.0	5.0	6.1	4.5	1.20	—
	A-47	21.0	5.0	6.1	—	—	—
	A-48	22.0	5.0	6.1	—	—	—

Repeated observations did not detect phosphorus in the lake water on any occasion except one when 0.005 p.p.m. phosphate was found at a depth of 22 m. Higher aquatic vegetation along the shore of the lake is very scarce.

Phytoplankton was practically absent on most occasions during the periods of observation but zooplankton occurred with occasional peaks of abundance of short duration. The zooplankton of the lake was described by Brehm (3).

Geologically the region belongs to the Laurentian Shield which "is underlain for the most part by a series of gneisses of great variation with which are associated crystallines, limestone, quartzites" (1). The gneisses in the Mont Tremblant Park area are of reddish and gray color often rich in quartz.

Materials and Methods

The samples of sediment used for the chemical and physical analyses reported in this paper were obtained on different occasions, and by three different sampling devices. Samples were obtained in the course of mapping and sounding operations during 1949 by means of a combination sounding weight which was attached to a calibrated chain. The exact location of these collecting stations was determined by transit from the shore of the lake at the time of sampling (Fig. 3). The numbers of the sounding stations on the map coincide with those of the samples made with the combination sounding weight. For example, C 55 was collected at station 55. Other samples of surface deposits were obtained in 1949 by means of an Ekman dredge. In 1950 all samples for chemical analysis and for almost all of the bacteriological observations were collected by a modified Sjöstedt sampler (18). The sampling tube of Pyrex glass is 45 cm. long, and is surrounded by a brass tube which ends at the lower extremity in a tapered ring of steel which can be removed by unscrewing. In order to obtain longer cores, the length of the apparatus was increased by inserting another length of brass tubing, containing a second glass tube between the first tubing and the tapered ring. The increased weight of the apparatus produced longer cores at most stations. At some localities it was found necessary to further increase the weight by surrounding both brass tubes with a length of heavy galvanized steel pipe of 5 cm. (2 in.) diameter. This made possible the collection of longer cores especially in the central part of the lake where sediments consist of deep layers of black sludge underlain locally by a stratum containing large amounts of gross plant detritus. No attempt was made to verify the amount of compression of the strata of the sediment in the collecting tube. At some stations the sampler proved unsatisfactory because of the deep surface layers of semiliquid sludge, which did not form a solid plug at the lower end of the sampling tube. The glass tubes were stoppered after removal from the sampler, and the color and other physical characteristics visible to the naked eye were observed for the different strata. Differentiation of strata was based on differences in color and texture as shown by the core in the tube. The thicknesses of the strata were measured to the nearest millimeter. Where the position of the layers showed irregularities at different sides of the tube the

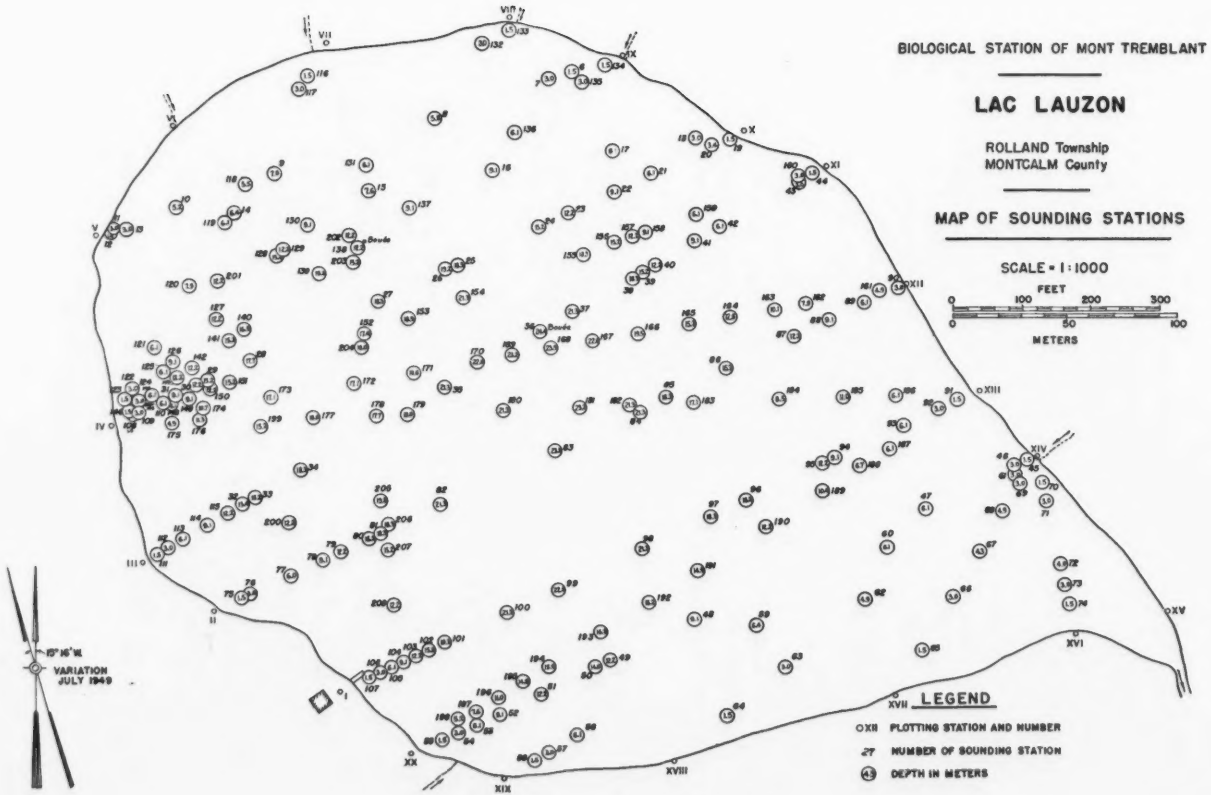

FIG. 3. Map of sounding stations.

average position was used for the measurement. The thickness of the lowest layer was indicated as ">" the length observed. By inserting a rubber piston into the lower end of the glass tube the core was pushed to the surface of the tube. When bacteriological samples had to be taken, these were collected first from the surface of the layer appearing at the top of the tube (4). After this the core was pushed up until the whole first layer protruded from the glass tube. It was then cut off and received in weighed Petri dishes for the determination of the water content (see below). The surface sediments at some stations were semiliquid, and had to be removed from the glass tubes by suction with a pipette. In some instances difficulty was encountered owing to the compression of air bubbles between the piston and the lowest end of the core. In some of the semiliquid samples, the compressed air was forced through different layers of the core to the surface, producing a disturbance of the stratification by which the sample was made useless for our purposes. In some instances, the differentiation between the various strata became difficult or impossible owing to the gradual changes in color and texture of the materials. From the portions of the cores so obtained, representative samples were taken for mounting on microscopic slides for later examination. These samples were preserved with formaldehyde after which suspensions in watch glasses were prepared and a few drops were mounted on microscopic

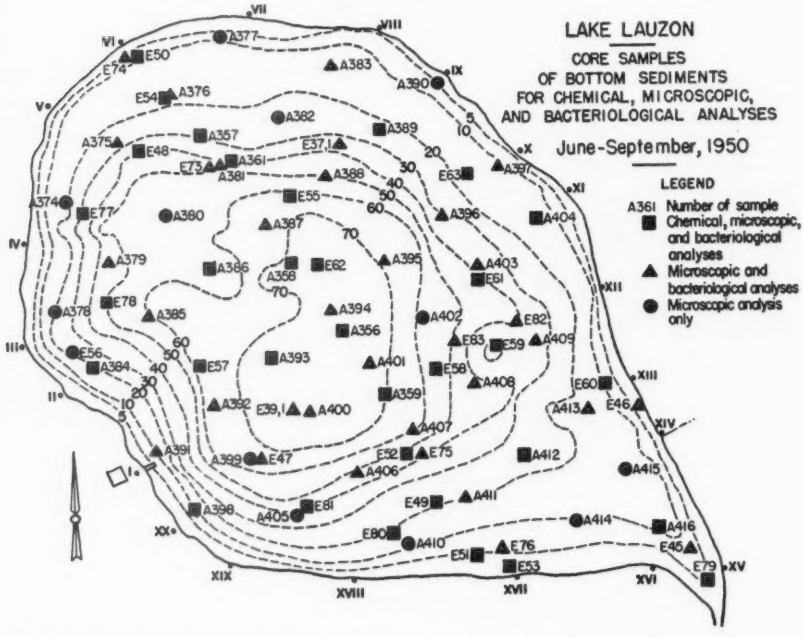

FIG. 4. Location of core samples for chemical, microscopic, and bacteriological analyses.

slides in glycerine jelly and covered with cover glasses. The edges of these were sealed with asphaltum. Three slides were prepared from each sample for microscopic examination.

All sediment samples for chemical analyses collected in the summer of 1949 were taken with Ekman dredges. Only the surface layer of each dredge sample was collected. It is realized that the samples obtained with the core sampler have the advantage of better definition of their vertical positions, although compression of the often semiliquid surface layers introduces here, also, an error in the measurements of the vertical distribution. The samples collected from the Ekman dredge contents were preserved with chloroform in the field, and sealed in hermetically closed jars. Although the addition of chloroform does not completely stop chemical changes in sediments it is believed that these changes are relatively small during the short time of storage.

Fig. 4 shows the position of the core samples whose strata were studied microscopically.

Chemical Methods

The wet material from the dredge was drained for one minute on filter paper supported by a copper gauze. A portion of the drained material was weighed and dried at 70-80° C. in an electric oven during 10 to 30 hr. The loss in weight was reported as the water content of the sample. The dried material was pulverized in a porcelain mortar and kept in a stoppered bottle with a few drops of chloroform as a disinfectant. The results of the analysis were given in percentage of weight relative to the dry material as obtained by the above procedure.

Loss on Ignition

Loss on ignition was obtained by incineration of the dry material in a porcelain dish or crucible during one hour in an electric furnace at 600° C. The ashes were conserved for mineral analysis. The determination of organic matter based on organic carbon content was not undertaken because of the lack of a dry combustion apparatus.

Organic Nitrogen

Organic nitrogen determinations were made by the Kjeldahl method. Samples varying in weight between 0.1 and 0.4 gm. were digested until total discoloration in concentrated sulphuric acid with selenium as a catalyst in the form of Hengar's granules. The distillate was received in 0.1 *N* or 0.02 *N* sulphuric acid.

Crude Lignin

Crude lignin was determined by the method described by Steiner and Meloche (20).

Mineral Analysis

Silica, aluminum oxide, iron, calcium, magnesium, and phosphorus were determined as follows. A 0.5 gm. sample of ash was fused with 3.5 gm. of

sodium carbonate. The melt was disintegrated with water in a porcelain evaporating dish and dissolved in hydrochloric acid. Silica was determined by the gravimetric method described by Scott (16). From the filtrate, iron, aluminum, and phosphate were precipitated twice with ammonium hydroxide. The hydrous oxides were dissolved in 1 : 5 sulphuric acid, the solution transferred to a 100 ml. volumetric flask, and brought to volume. On a 50 ml. aliquot, iron was determined volumetrically with 0.02 *N* potassium dichromate after reduction with stannous chloride. On a 20 ml. aliquot, aluminum and iron were precipitated with 8–hydroxyquinoline after the addition of a few drops of 3% hydrogen peroxide to prevent the precipitation of titanium; the precipitate was dissolved in 3 *N* hydrochloric acid and determined by bromometric titration with 0.1 *N* potassium bromide and 1 *N* arsenic trioxide. Aluminum was determined by subtracting from the hydroxyquinolate precipitate the amount of iron. Phosphorus was determined as ammonium phosphomolybdate in a 20 ml. aliquot following the volumetric method described by Pregl (15); 0.1 *N* or 0.02 *N* solutions of sodium hydroxide and hydrochloric acid were used according to the amount of precipitate obtained. The combined filtrates from the double ammonium hydroxide precipitations were evaporated to a volume of about 75 ml. Calcium was precipitated as oxalate and the precipitate titrated with 0.05 *N* potassium permanganate. Magnesium was precipitated in the filtrate with 8–hydroxyquinoline, and the resulting hydroxyquinolate determined volumetrically with 0.1 *N* potassium bromide and 0.1 *N* arsenic trioxide.

Physical Characteristics of the Sediments

Microscopic Observations

In the course of the summer of 1950, 64 cores were sampled representing all parts of the lake bottom. Figs. 3, 4, and 5 show the location of the sampling stations.

The results of the macroscopic study of a series of cores across the lake from shore stations VI to XVII are shown graphically in Fig. 6. A study of Fig. 6 shows that with the exception of a shallow area along the shores, the bottom of Lake Lauzon is covered by dark brown to blackish sludge locally mixed with fine sand.* This surface sediment whose thickness varies from 3.5 (E 49, 1-2) to more than 350 mm. (A 359) is generally of a semi-liquid consistence at the top but may become more solid in greater depth. In some areas of the lake (E 52, 1; E 49, 1-2; E 51, 1; E 61, 2; A 376, 1) the sludge becomes still more liquid while at some other stations it forms a colloidal suspension whose upper limits are often difficult to establish (E 60, 1; A 359; A 376, 1). The color of these suspensions varies from light brown to grayish. Observations made on different dates seem to suggest that the areas in which the surface sediment is represented by colloidal suspensions are well defined, and did not change their positions in the course of the summer observations. Thickness of the colloidal layer varies from a few millimeters

* *For the definitions of the size of the sand particles, see Sverdrup et al. (21).*

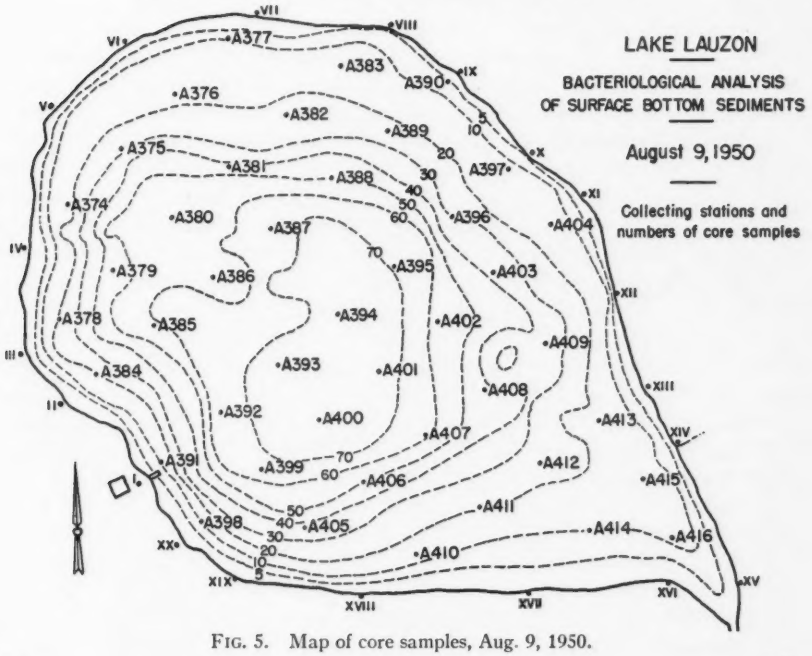

FIG. 5. Map of core samples, Aug. 9, 1950.

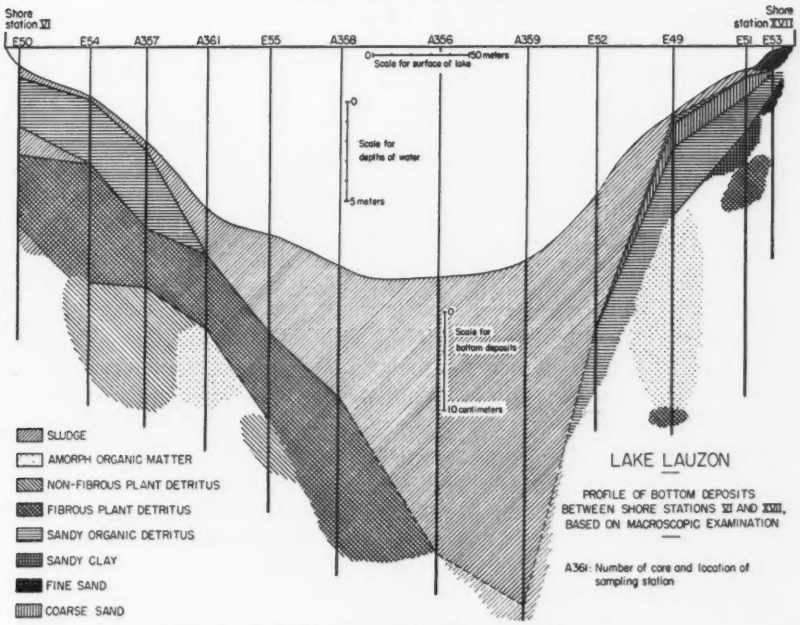

FIG. 6. Lake Lauzon, profile of bottom sediments.

to 70. The greatest thickness was found at a depth of 2.6 m. (E 60, 1) while a layer of only a few millimeters thickness was encountered at a depth of 11.5 m. At a depth of 3.8 m. (A 376, 1), the layer was only 25 mm. thick. It seems therefore that at least in Lake Lauzon there is some relationship between the depth of the lake and the thickness of the colloidal layer at the surface of the bottom sediments. Why this type of colloidal sediment should occur only locally is not known. An explanation for the local occurrence of these deposits may be found in the presence of outlets of ground water depleted of oxygen, holding ferrous salts in solution. Oxidation of the ferrous compounds at the point of mixture with well oxygenated lake water would result in the precipitation of ferric phosphate followed by that of ferric hydroxide (8, 9). At most stations the sludge is free of macroscopic fibers and the general appearance is amorphous. Whenever the layer exceeded a few millimeters in thickness, hydrogen sulphide was detected. The origin of this hydrogen sulphide was not investigated but might be sought in sulphates and organic matter, specifically chitin (19, 13) in the sediment. The oxidation of hydrogen sulphide by bacteria may cause oxygen depletion in the sediment and probably in the water immediately above it. The gas has a toxic effect on germs descending from the overlying water but its effect on anaerobes is apparently less than on aerobes (7). It is probable that, in the sludge, anaerobic conditions prevail even during periods of circulation. It is of interest that the bottom fauna is extremely scarce in almost all parts of the lake in which the surface bottom sediments consist of sludge. That anaerobic conditions may not extend to the uppermost layer, a few millimeters in thickness, is suggested by the increasing lignin content of the deeper parts of this sediment (Table IV). According to Steiner's observations (19) the decomposition of cellulose seems to be more intensive in anaerobic conditions, thus raising the relative content in lignin. The same author also attributes the formation of hydrogen sulphide to the decomposition of chitin as observed by him in the lower Lunz lake.

The surface sediment formed by the sludge is underlain in most parts of the lake by a stratum which was classified as sandy organic detritus. It can be seen in Fig. 6 that the latter stratum is confined to the more shallow parts of the lake down to a depth of 7.5 meters. It was not found underlying the sludge in the deeper part of the lake. This layer of sandy organic detritus is supported by a stratum of black sludge whose texture is similar to that of the sludge found at the surface of the bottom sediments of the lake (E50, 3), although the size of the particles seems to be smaller. In almost all other localities the sandy organic detritus was supported by a stratum whose texture was classified as fibrous plant detritus. In the cores taken in the central part of the lake (A 358) this material normally exceeded a thickness of 114 mm. The color of these deposits is normally dark brown, in some cases light brown to tan. Gross plant detritus in this stratum is found in all cores taken in the lake, and is represented mainly by remains of mosses. In the deepest part of the lake (A 356) it was impossible with the available apparatus to

obtain cores extending through the sludge into the layer of fibrous plant detritus which almost certainly underlies the former. In a few instances (A 354; A 357) a layer of plant detritus without macroscopically visible fibers was found below the fibrous plant detritus. It is possible that the former sediment represents a more thoroughly decomposed stage of the latter. Cores taken at some localities showed a stratum consisting of amorphous organic matter supporting the layer of fibrous plant detritus (A 361), while in other localities the amorphous organic matter is found above the fibrous plant detritus (E 49).

Variations in this relatively uniform pattern are found mainly in the shallow shore areas where often one or more of the strata just described are lacking. In these areas fine and coarse sand mixed with pebbles often replaces the sludge which may be found at lower depth at these localities.

Microscopic Observations

Slide mounts made of the bottom sediments taken from the core sampler were examined microscopically on the relative abundance of diatoms, sponge spicules, pollen, wood fibers, mosses, remains of plankton, tubes of worms, blue green algae, green algae, and leaves of phanerogams. Three different slides of each of the strata were examined under low and high power.

It may be observed generally that the horizontal distribution of the above-mentioned items is often erratic although trends can be shown in many instances. An analysis of the data demonstrates that in 50% of the surface sediments examined diatoms were common. Only in 20% of the samples were they abundant. In 20% of the mounts, diatoms were rare while in 4% of them they were totally absent. A correlation between these figures and the depth of the lake at different stations cannot be shown. In an ideally undisturbed lake, the relative abundance of the items composing the sediment might be expected to be proportional to the depth of the trophogenic layer of the water overlying the sediment, and to the depth of the water below the trophogenic layer as far as those materials are concerned which undergo decomposition on their way down to the lake bottom. In a natural lake however the descending particles will be affected by currents and turbulence. Their actions will be selective and dependent upon the size, shape, and specific weight of these particles. It may be expected also that particles with less buoyancy will present a more even distribution on the lake bottom than those which are more easily transported by horizontal water movements. The existence of considerable turbulence in the hypolimnion in Lake Lauzon was demonstrated in the course of the observations on temperature conditions in that lake (10). The apparently erratic distribution of the components of the surface sediments is therefore not surprising. A comparison of the relative abundance of diatoms in the surface sediments with that of deeper layers suggests that the sedimentation of diatom frustules was more intensive in the course of the formation of the surface sediments. In the next lower stratum which consists mainly of sandy organic detritus the relative abundance of diatoms drops sharply. In only 25% of all analyzed samples of this layer

are diatom frustules common. In about 40% they are rare while in more than 23% they are either very rare or absent. Inspection of the third stratum of a number of cores shows a further reduction in the relative abundance of diatoms. In almost half of the samples they were either very rare or absent. This trend continues in the fourth layer of most cores which consists mainly of organic matter mixed with fine sand and gross plant detritus. It is noteworthy that in deeper deposits, consisting of amorphous organic matter of unidentifiable origin, the relative abundance of diatom shells again increases. In these and in the following considerations bias is introduced by the fact that the number of samples of the lower strata was smaller than that of the upper strata. Thus the observations on the relative abundance of sediment components in the former are subject to greater error than those made on the relatively large number of samples of the upper strata. It is of interest to note that diatoms were absent or very rare where the surface sediment consisted mainly of fine sand. This again suggests selective action by currents and turbulence.

Sponge spicules were particularly abundant in all the strata of core E 51 in which only the upper stratum contained diatoms. In most samples sponge spicules are rare to common; only in a few instances are they abundant or even predominant. All stations at which sponge spicules are abundant to predominant are located in the southeastern extremity of the lake at which the present temporary outlet is found (Fig. 2). This is also the region of the lake where sandy deposits are predominant. It is therefore probable that the distribution of sponge spicules is also affected by prevailing currents in the lake as sponges may be found all around the lake in the shallow areas. In 50% of the surface layers examined, sponge spicules were rare. In only 16% were they common while in all other samples they were either very rare or absent. A similar picture is shown by the second layer in the cores where again in almost 50% of the samples sponge spicules were rare. The distribution is similar in the deeper strata. In only seven samples of all strata combined could sponge spicules be considered as being abundant while in five samples they were the most important component of the sediment. As a whole it seems that sponge spicules were relatively more abundant in the earlier history of the lake although their resistance to decomposition may be the cause for an apparent abundance in the older sediments.

In the most strata, pollen may be classified as common. In only three surface samples (7%) was it absent while in 21% it was classified as common and in 20% as abundant. As the identity of the pollen will be studied in the near future, more detailed data on its distribution will be presented at a later date.

In 33% of all surface samples, remains of mosses were present which were classified as common; in 9% of them this component was abundant. When examining the data on the deeper strata it will be noticed that at increasing depth the relative abundance of mosses in the sediment becomes greater so that in the second stratum 32% of the samples were classified as abundant in

mosses, and 33% of the third stratum received that classification. While only in one sample of the surface sediments were mosses predominant (somewhat over 2%) they were the most important component in 13% of the sediments of the third stratum. As these data indicate only relative abundance and not absolute quantities it is difficult to decide whether the increased relative abundance of remains of mosses in the deeper strata is due to quicker decomposition of the other organic compounds in the sediments or to other factors. Some of the cores present a very great abundance of remains of mosses (E 49; E 61; A 357; A 361; A 374; A 389). There is no apparent correlation between the occurrence of mosses and the location of the station although it seems that the cores of the deeper stations present a higher relative abundance of this component than those of the shallow stations.

Rests of plankton are more abundant in the upper layers of the cores. Only in one sample of the sixth stratum of one core (E 49, 6) could remains of plankton be classified as rare; they were absent in the only other sample of the same stratum. At still greater depth plankton remains were not found at all. Out of 46 surface samples examined rests of plankton were rare in 20, and common in 17. In eight of the samples this component was either absent or very rare while in one sample it could be considered as abundant. This picture is similar in the second and third layers but from the fourth layer downwards the relative abundance decreases sharply.

Bluegreen algae are rare in all surface sediment but are still found in surface samples taken at 12 meters (E 52, 1) depth. In Table III the frequency of

TABLE III

LAKE LAUZON. THE RELATIVE ABUNDANCE OF DIFFERENT COMPONENTS OF BOTTOM SEDIMENTS IN PER CENT OF THE TOTAL NUMBER OF SLIDES EXAMINED*

Item	Relative abundance and frequency, %					
	oo	o	x	xx	xxx	xxxx
Diatoms	15.8	11.1	23.8	34.9	14.3	—
Sponge spicules	4.7	12.7	47.6	22.2	7.9	4.7
Pollen	3.1	6.3	31.7	52.3	6.3	—
Wood fibers	66.6	6.3	31.7	4.7	6.3	—
Mosses	22.2	7.9	22.2	23.8	15.8	7.9
Remains of plankton	15.8	15.8	52.3	14.3	1.6	—
Tubes of worms	49.2	15.8	23.8	11.1	—	—
Unidentifiable amorphous organic matter	3.1	1.6	20.6	50.8	23.8	—

* oo absent xx common
o very rare xxx abundant
x rare xxxx predominant

relative abundance of the components of the bottom sediments of all strata is shown in per cent. The table shows that only sponge spicules and mosses were encountered as predominant components of the sediments. Of all the components considered, diatoms and mosses were most often classified as abundant. Remains of plankton and tubes of worms never represented an important part of the sediments. The unidentifiable amorphous organic matter may be classified as "dy" (14), and is almost always of light brown to yellowish color. It is probable that humic material constitutes the main chemical component of this amorphous organic matter. It is more commonly found in deeper layers which suggests high resistance in decomposition.

Chemical Characteristics of the Sediments

Table IV presents the results of the chemical analyses of surface sediments collected during the summer of 1949 with the Ekman dredge.

The water content of the sediments varied from 31 to 95% and was mainly correlated with the amount of sand present in the sediment and therefore with distance of the sampling station from the shore. Lowest water content was found at stations close to the shore line where most sand is deposited, and generally at depths less than six meters. At greater depths the water content increased rapidly from 70 to 95%. In spite of standard procedures and uniformity in sampling and treatment, considerable variations in the water content were found in the samples in the deepest parts of the lake. Samples collected at relatively short distances from each other (20 meters) may present differences in water content as high as 15%. At greater depths (17 to 24.4 meters), sand particles are only very rarely encountered, and then in very small amounts so that the differences in sand content can hardly explain the variation in water content of the samples.

The loss on ignition also varied widely in the lake but was largest in the deeper parts (K 4; E 3; E 20, 2). The highest value was found at station 14 with 75.0%. All the sediments with high percentage of loss on ignition were of the "sludge" type. As would be expected samples taken near the shore were rich in ashes owing to the mixture of sand in varying proportions. The percentage of loss on ignition varies from 2.5 (E 2, 2) to 75.0 (E 1, 2); the depth at the latter station was 15.8 meters. The slight variation in the ratio lignin: loss on ignition points to a relatively constant composition of the organic matter in the surface sediments throughout the lake. Of the 26 ratios calculated, 21 are between 30 and 50, three between 20 and 30. It might be stated generally that in the surface bottom sediments of Lake Lauzon lignin constitutes about one-half of the organic matter. There was no apparent correlation between the location of the sediment in the lake and the ratio lignin: loss on ignition. In some instances the ratio was affected by the presence of plant debris from the nearby shore. The nitrogen contents varied between 0.00 and 1.68% (K 8 and E 20, 2). In all samples with over 50% of loss on ignition the values for total nitrogen were relatively high with one single exception (E 5). None of the samples with very low loss on ignition contained appreciable amounts of nitrogen. Samples containing

TABLE IV

LAKE LAUZON. CHEMICAL ANALYSES OF SURFACE BOTTOM SEDIMENTS, JULY, AUGUST 1949

Date	Number of the station	Number of the sample	Depth of the lake in meters	Wet sediments		Dry sediments										Ratios		
				Water %	H_2S odor	Ashes	Loss on ignition, %	Lignin, %	Total nitrogen, %	SiO_2, %	Al_2O_3, %	Fe, %	Ca, %	Mg, %	P, %	Nitrogen / loss on ignition × 100	Lignin / loss on ignition × 100	Phosphorus / loss on ignition × 100
July 11	Station 72	E 28	4.2	33	Yes	?	3.4	—	0.07	73.8	9.38	1.61	1.66	0.61	0.04	2.03	—	1.16
" 18	" 186	E 16,2	5.8	83	No	63.75	36.2	15.2	0.73	46.9	6.70	0.87	1.17	0.24	0.05	2.02	42.0	0.14
" 12	" 186	K 8	6.1	31	No	97.40	2.6	0.7	0.00	80.6	7.80	0.87	1.02	0.04	0.01	0.00	28.8	0.38
" 1	Buoy 23	E 2,2	6.1	35	No	97.5	2.5	—	—	82.4	6.90	1.20	1.08	0.38	0.01	—	—	—
" 15	Station 136	E 13,2	6.1	70	Yes	83.31	16.7	2.5	0.31	61.5	9.66	1.43	1.69	0.54	0.07	1.85	15.0	0.42
Aug. 9	" 58	E 26,2	6.1	91	No	46.26	53.7	20.2	1.62	32.5	9.21	0.70	0.62	0.22	0.23	3.09	37.6	0.43
July 12	" 184	K 7	8.5	88	Yes	34.34	65.7	15.3	1.04	23.9	6.80	0.19	0.58	0.02	0.04	1.58	23.2	0.06
Aug. 4	" 163	E 24,2	9.1	83	Yes	70.90	29.1	11.6	0.87	56.1	7.88	1.45	0.96	0.09	0.12	2.99	39.9	0.41
" 25	" 185	AA 8	9.1	89	—	62.90	37.1	14.5	0.99	50.6	6.92	1.08	0.93	0.15	0.03	2.67	39.1	0.08
" 5	" 163	E 25,2	9.4	80	No	81.28	18.7	12.1	0.56	59.6	11.90	0.95	1.56	0.35	0.06	3.00	64.7	0.32
July 16	" 16	E 12,2	9.5	87	No	63.0	37.0	13.9	0.95	46.4	8.06	1.13	1.19	0.40	0.15	2.57	37.6	0.41
" 21	" 163	E 6,2	9.8	88	Yes	59.61	40.4	15.1	0.87	45.1	7.64	0.95	1.10	0.33	0.12	2.15	37.4	0.30
" 12	" 185	K 9	10.9	88	No	50.0	50.0	13.8	0.86	38.7	6.20	0.53	0.80	0.12	0.34	1.72	37.6	0.68
" 11	" 176	K 1	11.6	80	—	80.4	19.6	7.3	0.23	62.3	7.40	0.93	1.01	0.41	0.09	1.17	37.2	0.46
" 22	" 163	E 17,2	12.5	92	Yes	48.5	51.5	18.8	0.83	38.0	6.50	0.51	0.80	0.13	0.18	1.61	36.6	0.35
" 8	Buoy 24	E 5	13.1	89	—	49.4	50.6	13.2	0.85	37.4	6.56	0.95	0.70	0.03	0.42	1.68	26.1	0.83
Aug. 2	Station 163	E 22,2	14.3	85	Yes	73.27	26.7	11.3	0.73	56.2	7.55	0.83	1.13	0.14	0.11	2.62	42.3	0.41
July 26	" 163	E 18,2	15.2	92	Yes	45.31	54.7	23.3	1.30	35.2	5.71	0.74	0.63	0.15	0.14	2.38	42.6	0.26
" 1	Buoy 14	E 1,2	15.8	69	—	25.0	75.0	—	—	18.3	3.34	0.44	0.34	0.12	0.09	—	—	—
" 12	Station 183	K 6	17.0	90	Yes	41.14	58.9	22.3	1.03	30.5	6.26	0.19	0.51	0.02	0.04	1.75	37.9	0.09
" 11	" 179	K 2	18.0	93	Yes	45.1	54.9	25.9	1.25	35.2	5.74	0.67	0.62	0.17	0.30	2.28	47.0	0.55
" 27	" 163	E 19,2	18.3	89	Yes	55.36	44.6	16.5	1.00	36.4	8.19	3.87	0.92	0.24	0.15	2.24	37.0	0.34
Aug. 23	" 180	AA 6,1	21.3	95	Yes	43.47	56.5	24.6	1.64	35.1	5.86	0.66	0.44	0.11	0.21	2.90	43.5	0.37
" 3	" 163	E 23,2	21.3	—	—	49.19	50.8	23.1	1.48	37.8	5.61	0.57	0.75	0.06	0.13	2.90	45.3	0.26
July 11	" 180	K 3	21.3	81	Yes	39.20	60.8	29.4	1.07	28.5	7.53	0.67	0.55	0.17	0.30	1.76	48.3	0.49
" 12	" 182	K 5	21.3	83	—	74.04	26.0	11.6	0.57	56.2	9.56	2.64	1.44	0.31	0.17	2.19	44.6	0.65
" 1	Buoy 17	E 3	21.9	90	—	32.9	67.1	—	—	24.6	4.15	0.62	0.41	0.17	0.12	—	—	—
" 12	Station 181	K 4	23.1	92	—	40.1	59.9	28.7	1.43	30.2	6.42	0.40	0.44	0.02	0.23	2.38	47.9	0.38
" 28	" 185	E 20,2	23.1	93	Yes	45.35	54.6	26.7	1.68	34.2	5.20	1.40	0.56	0.15	0.25	3.08	48.9	0.46
" 7	Buoy 8	AA 2	24.4	93	Yes	42.2	57.8	26.3	1.24	32.7	5.24	1.16	0.56	0.36	0.22	2.15	45.5	0.38

intermediate amounts of organic matter presented also intermediate values for their nitrogen content. This relationship is well shown in the ratio nitrogen: loss on ignition (Table IV). The values for these ratios vary between 0.0 to 3.09. Of the ratios calculated for 27 samples, one is below one (K 8); eight are between one and two; and 15 are between two and three; only three are above three (E 26, 2; E 25, 2; E 20, 2). It was not possible to detect a correlation between the location of the sediment in the lake and the ratio nitrogen: loss on ignition.

The content of the samples in silica depends greatly on the location of the sediment in relation to the shore. The largest part of this silica has its origin in the sand grains which are more plentiful in the proximity of the shores. For the same reason there is a close relationship between the depth of the lake at the sampling station and the content of silica. In the deepest part of the lake 32.7% (AA 2) were found while the lowest value was registered in sample E 1,2 at a depth of 15.8 meters (18.3%).

The calcium content of the surface sediments varies between 0.34 and 1.69%. The highest value was found at a depth of 6.1 meters (E 13,2) and the lowest value at 15.8 meters (E 1,2). There is no consistent correlation between the calcium content and the depth. Neither was it possible to detect any correlation between the calcium content and that of the other chemical compounds analyzed. The ratio calcium: magnesium varies between 1.55 and 29.0. In 15 out of 31 samples however this ratio is between two and four. In only five samples did it exceed 20 (E 5; K 4; K 6; K 7; K 8). In four of these samples the ash content was below 50%. In all but one of the five samples the nitrogen content was slightly higher than the average for all samples (0.04). Values for magnesium varied between 0.02 and 0.61 (K 7 and E 28).

The amount of phosphorus in the sediments varied between traces (E 2,2) and 0.42 (E 5). The lowest values were found where the ash contents of the sediment were very high. High loss on ignition is not always accompanied by larger amounts of phosphorus e.g. sample K 7 with 65.7% loss on ignition contained only 0.04% of total phosphorus. In general however it may be stated that the sediments with the highest loss on ignition contained also the largest amount of total phosphorus. Of 11 samples containing less than 0.1% of total phosphorus only three presented more than 50% loss on ignition. This apparent relationship between phosphorus and loss on ignition seems to indicate that a considerable part of the total phosphorus in the sediments is present in organic form. There was no apparent correlation between the amounts of iron and those of phosphorus in the sediments. Total iron content varied between 0.19 and 3.87%. In 31 samples 10 contained more than 1% iron. The ratios between nitrogen and phosphorus varied considerably between 2.0 and 33.0. Eleven of the 21 samples presented ratios between 6 and 10; five were below six. Table V presents the average chemical composition of all surface bottom samples collected during the summer of 1949 with the Ekman dredge.

TABLE V

LAKE LAUZON. AVERAGE CHEMICAL COMPOSITION OF ALL SURFACE BOTTOM SAMPLES. SUMMER 1949

Item	Number of samples	Percentage of dry material
Loss on ignition	30	45.6
Crude lignin	26	17.08
Ashes	31	54.4
Total nitrogen	27	0.94
SiO_2	31	46.0
Al_2O_3	31	7.11
Calcium	31	0.87
Magnesium	31	0.21
Total phosphorus	31	0.14
Total iron	31	1.02
$\frac{\text{Lignin}}{\text{Loss on ignition}} \times 100$	26	37.4
$\frac{\text{Nitrogen}}{\text{Loss on ignition}} \times 100$	26	2.06
Ca : Mg	31	4.14
N : P	21	6.7
Percentage of water	29	84.9

During the summer of 1950, the strata of a number of cores were analyzed. Table VI presents the results available at the time of preparation of this manuscript. The location of these cores can be seen in Fig. 4. A 356 consisted of one stratum which was more than 290 mm. deep; the lake had a depth of 25.2 meters at the collecting station. The sediment was classified as black sludge and contained a considerable amount of unidentifiable organic matter of the "dy" type. Remains of plankton and diatoms were common but no tubes of worms were found. This sediment may be classified as dy-gyttja. Core A 357 was located at a depth of 4.8 meters. Its surface layer was not analyzed but consisted of black sludge and was 7 mm. thick. The second stratum (A 357,2) consisted of sandy organic detritus and had a thickness of 72 mm. Remains of mosses and unidentifiable organic matter and diatoms were abundant in this sediment. Remains of plankton and sponge spicules were common. When the chemical composition of this stratum is compared with the average values for the surface sediments of the whole lake it is noticed that the loss on ignition is relatively low (23.25%). The relatively high value of 60% for silica, as well as the proximity to the shore show that this is due to the presence of the sand, already noticed in the macroscopic classification of the sample. Values for nitrogen, phosphorus, and crude lignin are correspondingly low. Of interest is the relatively high content of calcium (1.39%), magnesium (0.39%), and total iron (1.24%). The higher value of calcium

TABLE VI

LAKE LAUZON. THE CHEMICAL COMPOSITION OF CERTAIN CORES, 1950.

Number of sample	Dry material, %									
	Loss on ignition	Ashes	Nitrogen	Crude lignin	SiO_2	Al_2O_3	Fe	Ca	Mg	P
A 356, 1	53.68	46.32	1.57	26.8	34.5	7.11	0.72	0.61	0.23	0.28
A 357, 2	23.25	76.75	0.64	8.7	60.0	7.45	1.24	1.39	0.39	0.103
, 3	37.92	62.08	0.99	15.7	47.5	7.30	0.92	1.10	0.30	0.165
, 4	52.09	47.91	1.24	21.3	37.1	6.20	0.61	0.75	0.17	0.186
A 358, 2	55.12	44.88	1.78	30.3	33.9	5.92	0.89	0.68	0.13	0.27
A 359	54.72	45.28	1.61	29.3	32.5	7.57	0.78	0.62	0.20	0.29
E 49, 2	0.95	99.05	0.039	0.49	86.5	5.55	1.58	1.15	0.41	0.009
, 3	2.50	97.50	0.052	0.42	78.7	6.50	2.44	1.23	0.65	0.011
, 4	40.14	59.86	1.50	14.1	45.6	7.88	1.17	0.99	0.33	0.118
, 5	65.57	34.43	0.67	22.8	22.7	7.92	0.36	0.81	0.06	0.201
E 51, 2	5.9	—	0.018	1.3	—	—	—	—	—	—
, 3	6.6	—	0.017	1.1	—	—	—	—	—	—
, 4	8.1	—	0.018	1.9	—	—	—	—	—	—
, 5	8.9	—	0.019	2.2	—	—	—	—	—	—
, 6	14.2	—	0.037	5.6	—	—	—	—	—	—
, 7	19.1	—	0.035	14.0	—	—	—	—	—	—

may be due to more intensive photosynthetic activities in this shallow region. The third stratum (A 357,3) of the same core had a thickness of 52 mm. and consisted of brown, fibrous organic detritus in which remains of mosses prevailed with rare remains of plankton and sponge spicules. Diatoms were common. Loss on ignition in this stratum increased considerably (Table VI) which is apparently due, in part, to an increase in crude lignin. The amount of silica decreased accordingly. Of the other elements the considerable increase in total phosphorus is of interest. In the fourth stratum (A 357,4), which consisted of nonfibrous plant detritus and had a thickness of over 120 mm., remains of mosses again predominated while diatoms and remains of plankton were rare, and sponge spicules were very rare. The data on Table VI show a further decrease in loss on ignition, nitrogen, crude lignin, and total phosphorus. Silica, aluminum oxide, iron, and calcium, decreased considerably in this deepest stratum. Core A 359 consisted of a single stratum taken at a depth of 10.6 m. Its thickness was over 350 mm. This sediment was classified as sludge and was covered at the time of collection by a colloidal suspension. Remains of plankton and diatoms were common in this sediment while sponge spicules were rare. It contained a higher than average amount of crude lignin (29.3%) and nitrogen (1.61%). The total phosphorus content was more than twice that of the average for the whole lake. Iron is lower (0.78%) in this sediment than on the average.

Of core E 49 the surface stratum was not analyzed. The second stratum E 49,2 consisted of fine greenish sand and was 6 mm. thick. Its low loss on ignition and low phosphorus content are noticeable, and are accompanied by a relatively high calcium content (1.15%). The loss on ignition increased in the deeper layers (E 49,3—E 49,5). The deepest stratum, which was classified as black amorphous detritus, was 200 mm. thick and was particularly rich in remains of mosses. Its crude lignin content is considerably above the average for the surface sediments of the lake. The phosphorus content of this stratum had a value of 0.201%. A comparison of the values for samples A 357 and E 49 reveals an increase in phosphorus content toward the depth. In the first case this increase was from 0.103 to 0.186%; in the second core the increase was from 0.009 to 0.201%. If the presence of phosphorus in the different strata of these cores were entirely due to the precipitation of ferric phosphate, as described by Einsele (8), it might be expected generally that the increase of total phosphorus would be accompanied by an increase in total iron. This is not the case however. The distribution of the iron is the reversal of that of phosphorus in cores A 357 and E 49. The highest values for phosphorus in both cores are found in the deeper layers where the values for iron are lowest. The explanation for this distribution might be sought in a greater availability of phosphates in the lake water at the time of the deposition of the lower layers. This hypothesis is the more attractive in view of the present phosphate depletion of the lake water. Lake Lauzon receives very little runoff from its small basin which is completely covered with vegetation. As was pointed out before, the lake does not have permanent inlets or outlets and is fed almost exclusively by ground water. It may therefore be expected that a replacement of phosphates, lost to the sediment by the precipitation of ferric phosphate, is inadequate. The well-defined thermocline, which is established in the early summer, and the late fall overturn, which may be expected in view of the well-protected position of the lake, are apt to produce long periods of stagnation with considerable oxygen depletion in this hypolimnium, owing to the presence of highly oxidizable bottom sediments throughout the lake. It may therefore be assumed that ferrous iron in the voluminous hypolimnium will cause the precipitation of all available phosphate as ferric phosphate as soon as an overturn occurs. This process was described in detail by Einsele (8), and it may explain the present oligotrophic condition of Lake Lauzon. That not all the phosphorus present in the sediments may be in the form of ferric phosphate is suggested by the invariable increase of total phosphorus, whenever the loss on ignition increased in the sediment. It seems apparent that at least part of the phosphorus is present in organic form. This hypothesis is further supported by the increase of nitrogen content in the lower strata. The low numbers of organisms found in the strata by Cooper, Murray, and Kleerekoper (4) are furthermore an indication that the decomposition of organic materials in these layers is very slow or may be even completely halted. The relative increase of crude lignin in the deeper strata, though, seems to point to more active decomposition of

cellulose. Steiner (19) found that cellulose is decomposed aerobically and anaerobically in the mud, but that the anaerobic decomposition is quicker, and that it is often accompanied by nitrate reduction.

There is no apparent correlation between the electric conductance of the surface sediments with the depth of the lake. In a number of cases the electric conductance is higher in the lower strata. (A 356,2; A 354,2; A 258,2; A 352,1; A 247,2). In four other cores however the reversal was true, and no differences were observed between shallow and deep strata.

Discussion of Results

Whereas one of the main purposes of the investigation, on which we are reporting, is to characterize lake bottom sediments from a regional approach, and to study the relationships between these sediments and the biological productivity of the lakes, final conclusions will not be presented before a number of other lakes in the geological region of Lake Lauzon have been studied as to the chemical composition of their bottom sediments. The data collected on the sediments of Lake Lauzon allow us, nevertheless, to discuss some of the possible relationships between the extremely oligotrophic nature of the lake and its bottom deposits.

One of the outstanding characteristics of the surface sediments of Lake Lauzon is their organic nature with an average loss on ignition of 45.6%. These sediments, which often contain hydrogen sulphide, show nitrogen and phosphorus contents (respectively 0.94 and 0.14%) which are high as compared to the scarcity of the elements in the lake water. The simultaneous occurrence of oligotrophy and organogenic bottom deposits, containing nitrogen and phosphorus in the above stated amounts, has not been reported on in limnological literature as far as the authors have been able to establish. This lack of reference is most likely not due to the rarity of these conditions, but rather to the scarce and incomplete information that has been gathered on the chemistry of lake bottom sediments. It has been generally accepted that oligotrophy is characterized by such a low organic production that the chemical and biochemical oxidation will keep pace with the production of organic matter in the lake. In other words, little organic matter will accumulate on the lake bottom, and this organic matter will consist of the more highly resistant chemical substances, which are a relatively small fraction of all organic matter produced. This also explains the slight decrease, if any, of dissolved oxygen in the hypolimnion during periods of thermal stratification.

The chemical characteristics of the sediments of Lake Lauzon do not answer this description of oligotrophy. Dissolved oxygen content of the hypolimnion has been occasionally very low. In spite of the low productivity of the lake, organic matter has accumulated in the bottom sediments, immobilizing nutrient elements like nitrogen and phophorus, which are evidently not returned to the nutrient cycles in the lake water. The problem now arises as to the cause of the accumulation of organic matter in an otherwise oligotrophic lake, and therefore of the loss of nutrients to the lake bottom sediments.

Accumulation of organic matter on the bottom is typical of dystrophic lakes, such as peat bog, in which the vegetation, in part, is characterized by its content in organic substances more highly resistant to decomposition. Partial decomposition in such environments leads to the accumulation of humic acids and other intermediate products of decomposition of humus. It is known that some of these substances have an inhibitory action on the growth of bacteria, which are the main agents in the decomposition of organic matter.

Lake Lauzon, however, is not a dystrophic lake and its bottom surface sediments are not derived from higher vegetation of the type found in bog lakes. It seems evident, nevertheless, that environmental factors are present in Lake Lauzon which slow down very considerably the decomposition of organic matter formed in the lake. Inhibiting factors would affect bacterial activity in a way similar to the one observed in dystrophic environments. Deficiency factors would act through the lack of nutrients for the hydrolyzing bacteria. Preliminary experiments showed that phosphorus availability may be an important factor in the rate of decomposition of the organic matter in the lake (4). The presence of inhibitors will have to be studied.

The results of our studies on the sediments of other lakes of the region will show whether the conditions found in Lake Lauzon are typical for oligotrophic lakes in this region.

References

1. ADAMS, F. F. Report on the geology of a portion of the Laurentian area lying to the north of the island of Montreal. Geol. Survey Can. Ann. Rept. (n.s.), 8 (Sect. J) : 7-184. 1896.
2. BIRGE, E. A. and JUDAY, C. The inland lakes of Wisconsin. The plankton. I. Its quantity and chemical composition. Wisconsin Geol. Nat. Hist. Survey, Bull. 64 : 1-222. 1922.
3. BREHM, V. The plankton of certain lakes in Mont Tremblant Park, Province of Quebec. In manuscript.
4. COOPER, B. A., MURRAY, E. G. D., and KLEEREKOPER, H. The bottom sediments of Lake Lauzon, Montcalm County, Province of Quebec. II. Bacteriological investigations. In manuscript.
5. COOPER, L. H. N. The rate of liberation of phosphate in sea water by the breakdown of plankton organisms. J. Marine Biol. Assoc. United Kingdom, 20 : 197-200. 1935.
6. COOPER, L. H. N. On the ratio of nitrogen to phosphorus in the sea. J. Marine Biol. Assoc. United Kingdom, 22 (1) : 177-182. 1937.
7. DÜGGELI, M. Hydrobiologische Untersuchungen im Piora-Gebiet. Bakteriologische Untersuchungen am Ritom-See. Rev. Hydrobiol. 3/4 : 65-205. 1924.
8. EINSELE, W. Über die Beziehungen des Eisenkreislaufs zum Phosphatkreislauf im eutrophen See. Arch. Hydrobiol. 29 : 664-686. 1936.
9. EINSELE, W. Über chemische und kolloidchemische Vorgänge in Eisen-Phosphat-Systemen unter limnochemischen und limnogeologischen Gesichtspunkten. Arch. Hydrobiol. 33 : 361-387. 1938.
10. KLEEREKOPER, H. Thermal conditions of Lake Lauzon in 1949 and 1950. In preparation.
11. KLEEREKOPER, H. A new apparatus for the study of sedimentation in lakes. Can. J. Zool. 30 : 185-190. 1952.
12. KLEEREKOPER, H. and GRENIER, F. Some chemical data on Mont Tremblant Park lakes. I. In preparation.
13. KLEIN, G. and STEINER, M. Bakteriologisch-chemische Untersuchungen am Lunzer Untersee. I. Die bakteriellen Grundlagen des Stickstoff und Schwefelumsatzes im See. Österreichische Botan. Ztg. 78 (4) : 289-324. 1929.
14. LUNDQVIST, G. Bodenablagerungen und Entwicklungstypen der Seen. Die Binnengewässer. 2. Stuttgart. 1927.

15. PREGL, F. and GRANT, J. Quantitative organic microanalysis. 4th English ed. The Blakiston Company, Philadelphia. 1945.

16. SCOTT, W. W. Standard methods of chemical analysis. 5th ed. D. Van Nostrand Company, Inc., New York. 1948.

17. SEIWELL, H. R. and SEIWELL, G. E. The sinking of decomposing plankton in sea water and its relationship to oxygen consumption and phosphorus liberation. Am. Phil. Soc. Proc. 78 : 465-481. 1938.

18. SJÖSTEDT, L. G. En orientiering över bottenförhallanda i Öresund och södra Östersjön. Lunds Univ. Årsskr. N.F., Avd. 2, 1923.

19. STEINER, M. Beiträge zur Kenntnis des Zellulose- und Chitinabbaues durch Mikroorganismen in stehenden Binnengewässern. Festschrift 75 J. Stelle Matutina II. Feldkirch. 1931.

20. STEINER, J. F., and MELOCHE, V. W. A study of lignous substances in lacustrine materials. Trans. Wisconsin Acad. Sci. 29 : 389-402. 1935.

21. SVERDRUP, H. U., JOHNSON, M. W., and FLEMING, R. H. The oceans their physics, chemistry, and general biology. Prentice Hall, Inc., New York. 1942.

22. WAKSMAN, S. A., CAREY, C. L., and REUSZER, H. R. Marine bacteria and their role in the cycle of life in the sea. Biol. Bull. 65 : 57-79. 1938.

STUDIES ON THE BIOLOGY OF THE MUSKRAT IN MANITOBA

PART I. OESTROUS CYCLE AND BREEDING SEASON[1]

By J. A. McLeod[2] and G. F. Bondar[3]

Abstract

A study of oestrus in female muskrats was conducted during the summers of 1950 and 1951 as part of a five year investigation of the biology of the muskrat in Manitoba. Two captive females examined daily by the vaginal smear method from June 30 to Aug. 10, 1950, inconclusively indicated an oestrous cycle length of about 30 days which agreed with the findings of Beer on Wisconsin muskrats. In 1951 10 female muskrats of various ages and of different lengths of time in captivity were examined daily beginning on March 13 and continuing until August 15. The data obtained at this time showed a considerable variation in the lengths of the oestrous cycles in different females or, even, in the same individual from time to time. The minimum time required for the completion of an oestrous cycle was found to be two days while the maximum time observed was 22 days. On the basis of 136 complete oestrous cycles studied, the modal time was found to be approximately four days and the mean time 6.1 days. The longer cycles found by ourselves in 1950 and by Beer are interpreted as representing pseudopregnancies and not oestrous cycles. Failure of muskrats to mate in captivity or in the wild during the latter part of the summer is attributed to sexual inactivity of the males and not the females.

Introduction

The investigation reported on herein is part of a broad study of the biology of the muskrat (*Ondatra zibethica alba*) in Manitoba, begun in 1947 and continued since that time. Many aspects of its biology such as food and shelter requirements, influence of weather, predators, parasites, and diseases, etc. have been studied synchronously and the results of these studies will be published later in this series. The present report deals with that portion of the work concerned with a study of breeding habits and reproductive potentials.

It is widely recognized that variations in reproductive rate may pronouncedly alter populations and population trends, particularly in a normally prolific species of short life span (McLeod (9)). It is important, then, to have a true evaluation of the reproductive potential of the muskrat in order to determine the extent to which this potential was being expressed and to what extent its expression was being altered by such extrinsic factors as weather, food, opportunities for mating, etc.

Muskrats have been mated successfully and have reproduced in captivity (personal communication with Dr. A. E. Woodhead, University of Michigan) but no records were published on the occurrence of oestrus or the length of the gestation period. Field observations by Errington (6), Enders (5), and Forbes and Enders (7) as well as by ourselves indicate that the gestation period of the

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muskrat is approximately 25 days and that a postparturitional oestrus occurs in from three to five days. However, these points have not been confirmed experimentally.

Prior to 1950 the literature contained no information on the time of occurrence of the first oestrus of the season, the length of the oestrous cycle, or the length of the potential breeding season of the muskrat. Several detailed studies had been carried out on other small mammals such as the albino mouse, albino rat, cotton rat, guinea pig, bank vole, rabbit, etc., the results of which were summarized by Young (16) up to 1941 and which form the basis for certain speculations regarding what might reasonably be expected in the muskrat.

In 1950, shortly after this portion of the investigation had been initiated there appeared a report by Beer (2) in which evidence was produced to indicate that the length of the oestrous cycle in Wisconsin muskrats was approximately 28.7 days, based on the study of seven specimens. His observations were not carried out over a complete breeding season so it was not definitely determined when the first and last oestrous periods of the season occurred. Beer, however, cited the sequence of the opening of the vagina and the occurrence of the first oestrus in nullipara of rodents and stated that, on this basis, in muskrats the first oestrus of the season should occur about the middle of March.

In order to verify this work and to establish any differences resulting from a more northerly climate or from subspecies identity it was decided to continue the observations. It was also hoped to obtain reproduction in captivity and thereby definitely determine the length of the gestation period and the time of the postparturitional oestrus if such occurred.

Procedure

In late June of 1950 two pairs of adult muskrats were captured in the field and each pair was established in an outside pen provided with nest box and small wire run. Neither female appeared to have produced young that season or to be pregnant at the time of capture. The activity of the reproductive system of each female was followed by means of the vaginal smear method of Stockard and Papanicolaou (12) using a pipette to collect the samples. Samples were collected thrice daily, diluted with physiological saline, smeared, flame dried, and stained with Harris' hematoxylin and eosin. Stained smears were examined with the aid of a research grade microscope using a magnification of 150. The cells were counted in three representative fields using a square-ruled ocular micrometer. Averages and percentage abundances of leucocytes, nucleated epithelial cells, and cornified cells were then worked out.

At the beginning of the 1951 observations on Mar. 13, there were available for study four females of less than one year of age which had been reared in the laboratory from an age of two weeks. In addition, there were two females which had been captured in the field the previous midsummer and

retained in the laboratory over winter. Each female was kept in a separate cage with nest box and wire run and was accompanied by a normal, healthy male which had been taken at the same time and maintained under like conditions. All animals used were part of a surplus originally kept for disease transmission work.

In order to detect any influence of long exposure to the captive environment, four pairs of adult wild muskrats were taken in Netley marsh, one pair being taken on each of the following dates: Apr. 30, May 30, June 1, and July 25, and kept under identical conditions for the duration of the experiment.

The room housing the animals was a basement one receiving direct sunlight only in the mornings. Here the temperature was moderately low and uniform with high humidity and good circulation of air. In order to vary the environmental conditions for part of the stock, one pair was exposed continuously to a 200 w. light at a distance of two feet. Exposure was begun on Mar. 26 and was continued for the duration of the experiment.

TABLE I

A BRIEF SUMMARY OF THE EXPERIMENTAL ANIMALS

No.	Age	Examination begun	Location	Remarks
1.V.	1950 kit, reared in lab.	Mar. 19	Animal room. Continuous 200 w. light, beginning March 26	Vagina opened Mar. 19
2.V.	Adult, caught in summer of 1950	Mar. 13	Animal room	
3.V.	1950 kit, reared in lab.	Mar. 18	Animal room	Vagina opened Mar. 18
4.V.	Adult, caught in summer of 1950	Mar. 13	Animal room	
5.V.	1950 kit, reared in lab.	April 3	Animal room	Vagina opened Mar. 19, copulated April 27
6.V.	1950 kit, reared in lab.	April 3	Placed in outside cage June 26	Vagina opened Mar. 19
1.N.V.	Adult? Caught May 30	June 4	Animal room	
2.N.V.	Adult? Caught June 1	June 6	Animal room	
3.N.V.	Adult? Caught April 30	May 5	Animal room	
4.N.V.	Adult? Caught July 25	July 30	Animal room	

The diet consisted of fresh carrots alternated daily with prepared fox and mink meal containing bone meal, meat scraps, alfalfa, etc. with one-third oatmeal added and fortified with wheat germ oil and fish oil containing vitamins A and D. This was supplemented on occasion with bread, cabbage, and lettuce.

Results

Examination of the two captive females in 1950 was begun on June 30, at which time both showed typical dioestrous smears. However, on July 3, one specimen showed a proestrous smear which was followed the next day by an oestrous smear in which the cornified cells comprised 90% and leucocytes 7%. This was followed by a metoestrous cell ratio the next day and a return to the normal dioestrous state. On July 8, there was a slight remission with 16% cornified cells but there was no further deviation from the dioestrous condition until Aug. 3 and 4, at which time the cornified cell count rose to 42% and the leucocyte count dropped to 56%.

The second female showed a proestrous condition for the first time on July 6, and this was followed the next day by full oestrus at which time the cornified cell count rose to 92%. In the first smear of the day large numbers of spermatozoa were present showing that copulation had taken place a few hours earlier at about daybreak. However, a pregnancy did not result. No further signs of oestrus were observed and the examinations were discontinued on Aug. 6. In both this and the previous case the smears following oestrus showed heavy mucification.

In 1951 the first smears were taken on Mar. 13, on the two captive adult muskrats. In the case of the female kits of the previous year the time of the opening of the vagina was noted and the taking of smears begun immediately or very soon thereafter. Smears were collected daily at 10.00 a.m. and the technique of the previous year was followed with the exception that here all smears were collected by means of fine wooden applicators instead of pipettes and care was taken not to excite or handle the specimen unnecessarily.

Careful examination of the smears gave the results listed in Table II. In the case of the first six specimens listed the first oestrous period recorded represents the first one of the season for the specimen concerned. Specimen No. 3. N.V. apparently had not produced a litter during the current year while in the case of 1.N.V., 2.N.V., and 4.N.V. it was impossible to determine this point with certainty without sacrificing the specimens. There is no way of knowing how many oestrous cycles the members of the latter group had experienced during the current season but one would judge it to be several. What is more important, each continued to cycle with some regularity after capture although the process may have been disturbed somewhat as a result of handling.

The tabulation below shows that approximately 86% of the oestrous cycles were completed in from two to nine days while the great majority of these required only from three to six days. The rather pronounced irregularity in

TABLE II

No.	Examination begun	First oestrus	Length of oestrous cycle*			Length of oest. period*		No. of cycles	Time* involved	% time in oestrus
			Max.	Min.	Av.	Max.	Min.			
1.V.	Mar. 19	Mar. 30	8	3	4.4	3	1	31	137	28.4
2.V.	Mar. 13	Mar. 14	19	2	6.4	12	1	21	134	30.6
3.V.	Mar. 18	Mar. 21	21	2	11.5	3	1	6	70	12.9
4.V.	Mar. 13	Mar. 27	22	2	6.9	9	1	18	125	31.2
5.V.	Apr. 3	Apr. 3	11	2	4.6	3	1	13	60	35.0
6.V.	Apr. 3	Apr. 7	21	3	7.1	3	1	15	106	27.6
1.N.V.	June 4	June 17	22	2	10.5	1	1	4	42	9.5
2.N.V.	June 6	June 10	16	2	6.6	9	1	7	46	32.6
3.N.V.	May 5	May 12	9	2	4.8	2	1	19	92	22.7
4.N.V.	July 31	Aug. 4	14	2	6.3	1	1	3	19	15.8

* *Time in days.*

the length of the oestrous cycle and the degree of oestrus exhibited by other small mammals as shown by Brambell, Rogers, and Rowlands (3); Clark (4); Long and Evans (8); Meyer and Meyer (10); Parkes (11); Young (15, 16); and Young, Meyers, and Dempsey (17) was also exhibited by the female muskrat. However, this is to be expected considering the complex hormonal balance involved as pointed out by Allen (1) and Turner (14).

Length of oestrous cycle in days	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Frequency	9	27	27	25	13	5	8	3	0	4	2	3	2	0	1	0	1	2	0	2	2

Complete oestrous cycles observed = 136.
Mean time per oestrous cycle = 6.1 days.

Fig. 1 is a graphic representation of the relative percentages of leucocytes and cornified epithelial cells in the vaginal smears of specimen No. 1.V. In this specimen, which was a laboratory reared kit from the previous year and, consequently, was approaching its first breeding season, the vagina opened on Mar. 19, and the first oestrous period occurred 10 days later. Following this there appeared to be a tendency to pass into oestrus rather regularly at intervals of about four and one-half days but the degree to which this was accomplished varied somewhat. The other specimens under observation showed even less uniformity in the lengths of the oestrous cycles than did specimen No. 1.V.

What degree of oestrus is necessary before mating will take place or before a pregnancy can result (if mating does occur) is not known. Only one mating was identified in 1951 and this was in specimen No.2.V. on Apr. 27. Like the single mating of the previous year, it occurred at the time of full oestrus.

Fig. 1 does not take into account the abundance of nucleated epithelial cells which remained very low during dioestrus but usually increased slightly during proestrus. In rare cases they about equalled the cornified cells in number during oestrus. One explanation of this phenomenon is that frequently the cycles are so short that the cornification can not reach completion and the precursors, the nucleated epithelial cells, are shed in their stead.

The degree of oestrus shown at different times undoubtedly is a reflection of the amount of oestrogen released into the system and this, in turn, will largely depend on the number of ova that are in the advanced stages of development or being released at the moment. In a species which normally releases from one to a dozen or more ova at a time it is understandable that the quantity of oestrogen released at one time might vary and, at times, be less than maximum. Other specimens under examination during the year showed very similar oestrous cycles so that specimen No. 1.V. is taken as being moderately typical. Some long oestrous cycles of up to 22 days were recorded but they were very rare. The longest continuous oestrous period was recorded for specimen No. 2.V. and lasted 12 days. This was exceptional, however, and in the great majority of cases the oestrus lasted only from one to two days.

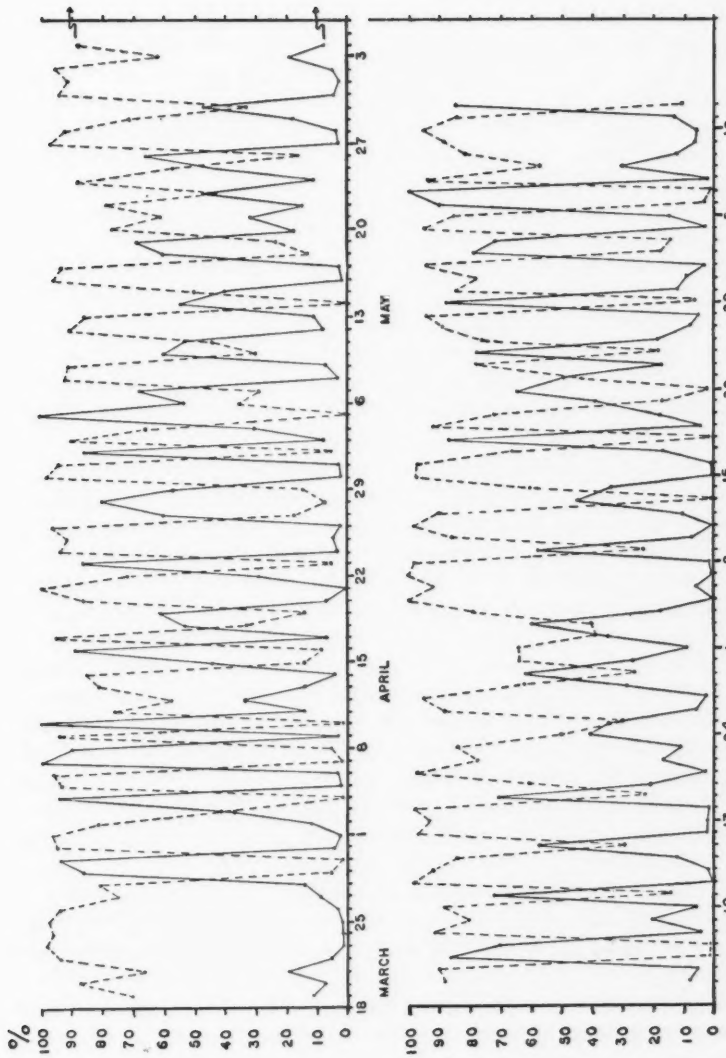

FIG. 1. Relative abundances of leucocytes (- - -) and cornified cells (——) in vaginal smears of specimen No. 1.V. Stilbestrol injections begun July 19, 1951.

How long oestrus continues to recur in the late summer or autumn was not determined but no specimen had passed into an oestrus when the counts were discontinued on Aug. 14.

The longer oestrous cycles of 28.7 days established by Beer (2) and the inconclusive one of 30 days suggested by our experiments of 1950 could have been normal for the specimens under observation. But, in the light of our more recent work, the longer cycles might be interpreted as the premature falling off of sexual activity. A more logical explanation appears to be that these do not represent normal cycles but pseudopregnancies induced by handling the specimens or by cervical stimulation during the collecting of vaginal material as shown by Turner (14).

There appears to be a considerable difference in the time and the degree of greatest sexual activity between members of the two sexes. Male muskrats in Manitoba, both captive and wild, show an increase in testicular size and sexual activity with the approach of warmer weather in the spring and the maxima correspond to, or follow shortly after, the appearance of open water. It is much more pronounced in wild specimens but is also shown by captive specimens and the only captive mating obtained in 1951 was on Apr. 27. Following this sudden and intense upsurge there is a progressive and moderately rapid loss of sex interest by the males.

Examination showed that, as might be expected, there was a sharp increase in testis size with the approach of the mating season but this was followed in about three weeks by a progressive decrease. By the middle of August the testes had shrunken to about one-half their maximum size. Coupled with this was a reduction in the production of spermatozoa although some living spermatozoa were present in the epididymes examined as late as the third week in August. At what time the males become incapable of fertilization is unknown and attempts to recover viable spermatozoa for artificial insemination about midsummer by means of an electric shocking device were unsuccessful. The testes of captive specimens showed only slightly more rapid atrophy than those of wild individuals but the four males brought directly from the field refused to mate with oestrous females.

In an effort to check the reliability of the vaginal smear method of determining the time of oestrus in the female and to stimulate sexual activity in the males and thus obtain mating in captivity, specimens were injected intramuscularly with sex hormones as shown in Table III.

The injection of stilbestrol did not appreciably alter the oestrous cycles in the females as shown by the vaginal smears, apart from the possibility of a slight intensification (Fig. 1). Some increase in testicular size was noted in the males in response to rather large injections of testosterone but it was not sufficient to result in spontaneous mating or even a show of sexual activity. The presence of a tank of water might have facilitated mating since it may usually occur in water in nature as suggested by Svihla and Svihla (13) but our earlier observations and those of others indicate that the presence of water is not essential.

TABLE III

Date	Dosage	Specimen numbers				
Females—stilbestrol						
19/7/51	0.003 mgm.	1.V.	2.V.	1.N.V.	2.N.V.	
20/7/51	0.003 "	"	"	"	"	
21/7/51	0.006 "	"	"	"	"	
23/7/51	0.006 "	"	"	"	"	
Males—testosterone						
19/7/51	0.25 mgm.	1.V.	4.V.	1.N.V.	3.N.V.	4.N.V.
20/7/51	0.50 "	"	"	"	"	"
21/7/51	0.50 "	"	"	"	"	"
23/7/51	0.50 "	"	"	"	"	"
23/7/51	1.00 "	"	"	"	"	"
27/7/51	0.50 "	"	"	"	"	"
27/7/51	0.75 "	"	"	"	"	"
27/7/51	1.25 "	"	"	"	"	"

Field observations in Manitoba covering five complete breeding seasons offer extensive evidence that young muskrats are rarely born before May 18. Out of several hundred litters examined in the field the earliest date of birth established was May 11, in 1950. Following May 20, in a normal year, the exact time varying with the time of breakup, there is a pronounced upsurge in the production of young within the next two weeks on the average. With the exception of the summer of 1950 the greatest number of new litters was found at this time.

Considering the arrival of new litters by weekly intervals it is normally seen that, following the first upsurge in rate, the rate drops very low and then almost exactly one month after the first there is a second but smaller upsurge. This phenomenon appears to be repeated with a smaller third and an almost negligible fourth, each occurring about a month following the previous one. Only very rarely are young born after August. One such exception was on Sept. 20, 1950, in Delta marsh.

Such observations tend to support rather strongly the contention of an oestrous cycle in the female of about one month. However, the information presented here disproves this. The only explanation of the phenomenon which can be advanced at the moment is that if a female reproduces at all during the season she becomes pregnant during the more intensive period of the mating season and not at a later date except in rare cases. It would appear that a portion of these females mate again producing second litters about one month after the birth of the first. A progressively diminishing number of these females would then produce third and fourth litters at about monthly intervals.

There is not more than a slight suggestion of even seasonal pairing among muskrats and, certainly during the more active portion of the breeding season in the spring polygamy appears to be the rule. Our laboratory findings show that the female become sexually active earlier in the season and may pass through three or four oestrous periods before the male comes into sexual activity. It is also reasonably certain that the female would have an opportunity to come into oestrus two or even three times during the period of heightened sexual activity of the male.

Even under conditions of moderately low population density this should allow an opportunity for almost every female to be mated, which in actual practice appears to be the case. The progressive decline in the numbers of second, third, and fourth litters of the season appears not to be attributable to the absence of oestrus or a length of the oestrous cycle in the female but to a progressive decline in the sex activity of the males with the approach of warmer weather. Superficial evidence suggests that lactation and the suckling of young do not interfere with the production of a second pregnancy.

The reproductive rate of the muskrat, then, appears to depend almost exclusively on the sexual activity of the male rather than on that of the female. If that of the males equalled the present season and intensity of that of the females the reproductive rate would be doubled or even trebled. However, this may serve as a natural guard against physical exhaustion and the presence of very immature individuals at a time when winter is approaching.

References

1. ALLEN, EDGAR, *et al.* Sex and internal secretions. The Williams and Wilkins Company, Baltimore. 1939.
2. BEER, J. R. The reproductive cycle of the muskrat in Wisconsin. J. Wildlife Management, 14(2) : 151–156. 1950.
3. BRAMBELL, F., ROGERS, W., and ROWLANDS, I. W. Reproduction of the bank vole (*Eutomys glareolus* Schreber). Phil. Trans. Roy. Soc. (London), B, 226 : 71–98. 1936.
4. CLARK, F. H. The oestrous cycle of the cotton rat. Contrib. Lab. Vert. Genetics, Univ. Michigan, 2 : 1. 1936.
5. ENDERS, R. K. The corpus luteum as an indicator of the breeding of muskrats. Trans. Fourth N. Am. Wildlife Conference, pp. 631–634. 1939.
6. ERRINGTON, PAUL. The breeding season of the muskrat in northwest Iowa. J. Mammal. 18(3) : 333–337. 1937.
7. FORBES, T. R. and ENDERS, R. K. Observations on corpora lutea in the ovaries of Maryland muskrats collected during the winter months. J. Wildlife Management, 4 (2) : 169–172. 1940.
8. LONG, J. A. and EVANS, H. M. The oestrous cycle in the rat and its associated phenomena. Mem. Univ. Calif. 6 : 1–113. 1922.
9. MCLEOD, J. A. A consideration of muskrat populations and population trends in Manitoba. Trans. Roy. Soc. Can., V, 44 (111) : 69–79. 1950.
10. MEYER, B. J. and MEYER, R. K. Growth and reproduction of the cotton rat, *Sigmodon hispidus hispidus* under laboratory conditions. J. Mammal. 25 : 107–129. 1944.
11. PARKES, A. S. Observations on the oestrous cycle of the albino mouse. Proc. Roy. Soc. (London), B, 100 : 151–170. 1926.
12. STOCKARD, C. R. and PAPANICOLAOU, G. N. The existence of typical oestrous cycle in the guinea-pig with a study of its histological and physiological changes. Am. J. Anat. 22 : 225–283. 1917.

13. SVIHLA, A. and SVIHLA, R. D. The Louisiana muskrat. J. Mammal. 12 : 12–28. 1931
14. TURNER, C. D. General endocrinology. W. B. Saunders Co., Philadelphia and London. 1949.
15. YOUNG, W. C. The vaginal smear picture, sexual reproductivity and the time of ovulation of the guinea-pig. Anat. Record, 67 : 305. 1937.
16. YOUNG, W. C. Observations and experiments on mating behaviour in female mammals. Quart. Rev. Biol. 16 : 135–156, 311–335. 1941.
17. YOUNG, W. C., MEYERS, H. I., and DEMPSEY, E. W. Some data from a correlated anatomical, physiological and behavioristic study of the reproductive cycle in the female guinea-pig. Am. J. Physiol. 105 : 393–398. 1933.

THE IMPORTANCE OF THE LOCUS OF APPLICATION ON THE EFFECTIVENESS OF DDT FOR THE HOUSE FLY, *MUSCA DOMESTICA* L. (DIPTERA: MUSCIDAE)[1]

By R. W. Fisher[2]

Abstract

DDT–benzene droplets, 1.5 ml. and up, produced by a 0.25 ml. syringe combined with a microfine glass tube and a rocking microtome (to regulate the plunger movement) were collected, evaporated on water, and applied as saturated droplets or crystalline masses to 16 different areas of the integument of adult *Musca domestica* L. males harmlessly immobilized in cellulose tape holders.

Crystalline DDT on the pulvilli of one leg or injected into muscle was ineffective. DDT in benzene was more effective than crystalline DDT. Effectiveness increased as loci of application approached the body or head; was enhanced by pulsatile membranes beneath loci; was greatest and most rapid when the labella were treated; was increased as the area of intact integument surrounding the locus was increased; but was not enhanced by additional cuticular waxes in the benzene solvent.

Introduction

An important property of the insecticide DDT is its ability to kill insects by contact with the intact cuticle. Because of this property, it is often applied on structural surfaces which then become toxic to insects contacting them. For instance, house flies may acquire a toxic dose by walking on a DDT-treated surface, and because the tarsus alone is in contact with it, it is assumed that this is the point of entry for the poison. Yet, flies may also be killed by the application of DDT to the mesonotum, and by flying through a mist of fine DDT-containing droplets which impact mostly on the wings. Is the cuticle then uniformly susceptible to the contact action of DDT, or are there differences associated with different areas of the insect integument? If such differences exist, they may afford a further approach to the mode of action of DDT which, despite extensive study, remains elusive.

This study is concerned with the mortalities produced by the application of uniform dosages of DDT to discrete areas on the cuticle of the housefly. The results show large differences in mortality depending on the locus of application and the size of the cuticular area available to the insecticide, and lead to the concept of the cuticle as a tissue actively involved in the insecticidal action of DDT.

Methods

Microdrop Apparatus

(*a*) *Construction*

The apparatus is shown in Fig. 3. A 0.25 ml. tuberculin syringe was mounted vertically on a Cambridge rocking microtome so that the plunger

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was advanced by the distal end of the lever arm as the ratchet wheel was turned. The syringe was fitted with a 22-gauge hypodermic needle and the union made benzene-tight by three successive layers of liquid solder. The point of the needle was clipped off and a piece of soft glass tubing (3 mm. diameter, 1 mm. bore) fused to it in a small flame. This joint was reinforced by "Hi-Pyseal" cement to prevent cracking on cooling. The glass rod was then drawn out to a fine capillary and bent in two places so that the open end pointed downwards when the syringe was in operation.

(*b*) *Operation and Calibration*

The syringe was filled by removing the plunger and pipetting the liquid into the barrel. The plunger was returned, all air expelled, the syringe mounted, and the plunger advanced by the ratchet until the lever arm was horizontal. The syringe was filled with water and the ratchet wheel turned through 20 complete revolutions. The expelled water was weighed and the weight divided by the number of ratchet divisions. In this way it was established that the device delivered 0.0000156 ml. per ratchet division. Uniformity of successive droplets was examined by drawing a large number of them directly from the end of the syringe into a long capillary tube. As the individual droplets were separated by air in the capillary, they could each be drawn in turn to a chosen portion of the tube and measured on a fine scale. Thirteen of 18 such droplets measured exactly 13 mm., and the remainder were within the range 12–13.6 mm.; the error for the 18 droplets was 0.23%.

(*c*) *Production and Collection of DDT–Benzene Droplets*

Solid DDT (1-trichloro-2,2-*bis* (*p*-chlorophenyl)-ethane) cannot be applied in measured doses to selected regions of insect integument, and dilute solutions of DDT in benzene spread over excessively large areas. Consequently, it was necessary to reduce the amount of solvent to a minimum. Solutions of high concentration could not be dispensed from the syringe without crystallization taking place. Therefore, droplets of a 1% solution were produced and allowed to evaporate to a highly viscous state on the surface of water. The procedure was as follows:

A solution of "Aerosol"* surface active agent was made to contain one drop of a 1% stock solution in 100 ml. of distilled water. A shallow glass dish with a piece of mirror fastened to the bottom was partly filled with the "Aerosol" solution so that the water surface was relatively flat in the center and sloping upward sharply at the margin of the meniscus. The mirror bottom reflected light up through the droplets and gave them a definite outline.

By raising this collecting dish the operator could take (with the aid of a binocular microscope) a droplet of the 1% solution of DDT in benzene from the syringe capillary and deposit it on the sloping surface of the meniscus. Here the droplet spread out on the water in a triangular film and evaporated quickly to a tiny bead as it gravitated toward the center of the dish. Successive droplets could be deposited at 5- to 10-sec. intervals so that finally a large

* *A surface-active agent—American Cyanamid Co.*

number of supersaturated droplets of DDT in benzene could be collected in one dish. These viscous beads were mutually repelled and did not coalesce. The concentration of "Aerosol" was critical. Too much caused the droplets to stand up as delivered, evaporate too slowly, and often coalesce; too little allowed the droplets to spread too quickly and break up. Drafts, dust, or grease from the fingers disrupted the forces in the collecting surface or caused crystallization of the DDT.

The concentration of DDT in the supersaturated solution was determined by weighing a large number of drops picked up on a glass rod and reweighing after crystallization of the mass by seeding. The drops contained 89.74% DDT by weight. Knowing the volume delivered, it was calculated that an advance of one notch on the ratchet wheel of the microdrop machine delivered a droplet containing 0.167 μgm. of DDT. In most tests, the ratchet was advanced six notches to give droplets containing approximately 1.0 μgm. of DDT.

(*d*) *Application of the Supersaturated Benzene Solution of DDT*

The droplets were applied to the insect by means of glass applicators. Applicators were made from soft glass rod or tubing 3 mm. in diameter. Each was drawn down at one end to a flexible filament 1 mm. long and a few microns in diameter.

A droplet was removed from the water surface by touching the tip of the applicator to it and jerking it upward to overcome the surface attraction. It was then touched to the treatment site and the applicator slowly withdrawn. The droplet settled on the cuticle of the fly, spread slowly to about twice the original diameter, and disappeared into the cuticle, leaving only a dark area to mark the spot.

To prevent the flies from transferring the applied DDT to other parts of the body, simple holders of cellulose tape were designed.

Two pieces of cellulose tape (½ in. width) 18–20 mm. long were placed sticky sides together to give an overlap of 7 mm. This was then stuck to a microscope slide and the overlap region pierced by a tool which turned back a flap (Fig. 1*b*).

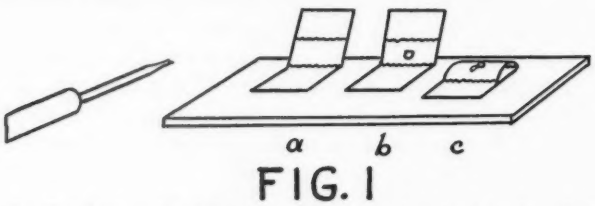

FIG. 1

FIG. 1. Punching tool and the three steps followed to produce the cellulose tape holder which exposes the leg of the fly for treatment; actual size.

To expose a leg, a circular hole 0.5–1.0 mm. in diameter was made with a cylindrical tool with a sharp concave slanting face. The flap was turned upward and pressed back firmly. The leg of the fly was inserted through the

hole and the sticky portion brought over the body and stuck down to the slide, thus enclosing the fly in a nonsticky half cylinder of tape (Fig. 1*c*).

Similarly, with tools of different size and shape, holes were made to accommodate the head, abdomen, or wing (Fig. 2). For treating the dorsal thorax, the tool shown in Fig. 4 was used, and the head and wings were firmly held by the tape, leaving the thorax exposed as shown in Fig. 5.

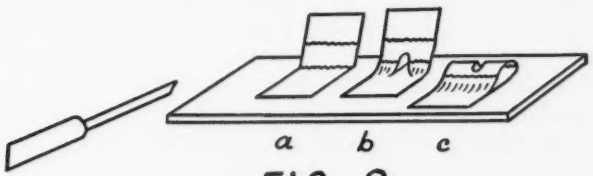

FIG. 2

FIG. 2. Punching tool and the three steps followed in making the holder which exposes the head or abdomen of the fly for treatment; actual size.

Rearing Methods

A culture of *Musca domestica* L. was established with pupae from a susceptible strain from the Dominion Fumigation Laboratory in Montreal, P.Q.

(*a*) *Larvae*

The method of Hafez (4) as standardized by Fisher and Morrison (2) was further modified to rear uniform test insects. Eggs laid during the night were washed and 0.4 ml. allowed to settle in the bottom of a graduated vial. These were then suspended in 16 ml. of distilled water and 2 ml. of the suspension (300–500 eggs) pipetted onto each culture pad.

Snug-fitting pads of cellucotton (about 72 layers thick) placed into the bottom of pint milk bottles were uniformly wetted with 60 ml. of pasteurized homogenized milk. These served as culture units. Light cotton stoppers were used for the first three days to prevent drying of the medium, after which 3 in. of fine sawdust was added to the top of each pad. A good culture yielded 200–250 pupae. The life cycle from egg to adult took eight to nine days.

Only the nonsterilized cellucotton gave satisfactory physical conditions in the larval medium. Temperatures above 28–29° C. forced larvae into the upper layers and prevented full growth while lower temperatures lengthened the larval period so that the proteolytic organisms predominated and made the medium foul and even toxic. A darkened rearing cabinet seemed to promote better larval feeding.

(*b*) *Pupae*

Pupae were easily and cleanly separated from the sawdust by a modified Goodhue and Linnard (3) separator built around a small vacuum cleaner. A lantern glass fitted with a fine screen bottom was half filled with the sawdust and pupae from the culture bottles. Suction was applied to the top and the air entering through the screen carried the sawdust upward and out, leaving the pupae clean. The shape of the glass created a turbulence and prevented the pupae from being drawn out with the sawdust.

The pupae were then screened through barley screens having 7/64-, 6½/64-, and 6/64-in.-wide slots, respectively. Those which did not pass or stick in the second screen were rescreened several times and retained for test work. Those from on top of the first and third screens were kept to stock breeding cages. Pupae used for tests averaged 22 mgm. each in weight and 40% of them emerged as males. Emergence exceeded 98%.

(*c*) *Rearing-stock Adults*

Adult stocks were kept in metal-bottomed screen cages 12 in. × 12 in. × 12 in. which could be taken apart for washing. A battery of cages handled as described by Fisher and Morrison (2) gave a continuous supply of test insects. Adults were fed on diluted milk and given milk-soaked pads on which to oviposit. A crack in the center of the pad encouraged the flies to concentrate the eggs in that spot.

(*d*) *Test Adults*

The sexes were separated by the method of Fisher and Morrison (2), and males were used in all tests. The males were retained and fed for five days in small cloth covered cages. From these cages they were shaken gently into a pint milk bottle fitted with a cork stopper having a ½ in. hole occluded by a sliding metal door. By swinging the bottle through a short arc five to six flies could be propelled through the open door into a 7 ml. vial. Random selection of flies for treatment was made by selecting these vials at random.

The flies were lightly anaesthetized with carbon dioxide and introduced immediately into cellulose tape holders. With practice five flies could be placed in holders in two to three minutes. Forceps of very light weight, springy metal permitted safe handling of the flies regardless of the finger pressure used.

Experimental

Effect of Crystalline DDT on the Tarsus

Because flies normally contact a DDT-treated surface with the tarsus, it has been generally assumed that the tarsal segments and particularly the pulvilli are highly effective areas for the sorption of DDT. A number of trials were conducted to test the validity of this idea.

By means of cellulose tape bandages, 0.067 μgm. of crystalline DDT was applied and held firmly in contact with various parts of the tarsus of individual flies for 24 hr. No mortality occurred in 30 flies treated in this way, nor was there any mortality when 15 additional flies were treated with the same amount of DDT pressed firmly into the distal tarsomeres and held in contact for 24 hr. Only 1 fly out of 14 died when the same dosage of DDT was applied to the pulvilli alone; a copious secretion was produced between the pulvillar pads by a few flies but not by the fly that died. Mortality resulted only when the flies were permitted to scratch, with the pulvillus and distal tarsomeres of one hindleg, a strip of scotch tape covered with fine crystals of DDT. Forty flies treated in this way showed large accumulations of DDT crystals matted on the setae on the ventral surface of the distal segments, and 100% mortality occurred in 24 hr.

PLATE I

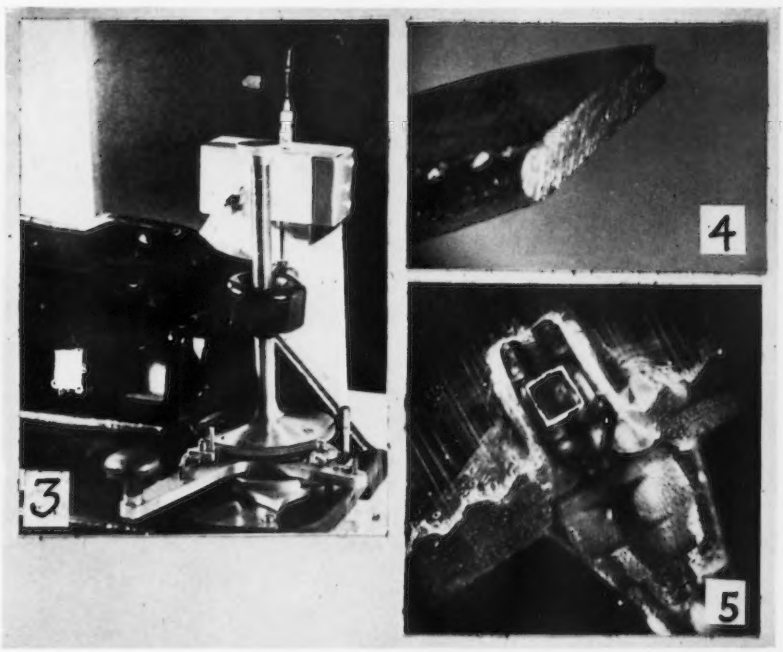

FIG. 3. Ratchet propelling device and syringe holder on the converted Cambridge rocking microtome.

FIG. 4. Photomicrograph of the end of the tool for punching the hole in the tape to expose the thorax for treatment; scale: 1 cm. = 1 mm.

FIG. 5. Photomicrograph of the mesonotum of a house fly showing the large "island" cut in the integument.

The Effectiveness of DDT on Various Integumental Areas

Supersaturated droplets of DDT in benzene, produced by the method already described, were applied to discrete areas of the integument. The dosage applied to each locus was 1 μgm. of DDT. The flies were immobilized with carbon dioxide and placed in the cellulose tape holders already described. When applied, the droplets spread over a small area of the cuticle and disappeared into the cuticle in a very short time. Attempts to crystallize the DDT on the surface of the cuticle by seeding were unsuccessful.

(*a*) *Tarsal Segments*

One microgram of DDT was applied on one of the five tarsomeres or on the pulvillus of individual flies. Ten replicates of 15 flies were used for each of these sites of application. The results show the relative efficiency of the pulvillus and the tarsomeres in promoting the toxic action of DDT. After 24 hr. the number of flies dead was as follows:

Treated on tarsomere 1	66
tarsomere 2	51
tarsomere 3	44
tarsomere 4	44
tarsomere 5	41
pulvilli	36
Untreated	0

Necessary differences were 13.79 for $P = 0.05$; 18.30 for $P = 0.01$.

Application of DDT to the first tarsomere caused significantly higher mortality than application to the second tarsomere and very significantly higher mortality than application to the remaining distal parts. Applications to the second tarsomere produced significantly higher mortalities than applications to the pulvilli, but there was no significant difference between the third, fourth, and fifth segments and the pulvilli.

(b) Pulvilli and Leg Regions Proximal to the Tarsus

Ten flies were used in treating each of the sites noted below. The test was replicated 10 times to make 100 flies per area treated. After 24 hr. the number of flies dead out of 100 treated with 1 μgm. DDT was as follows:

Treated on coxotrochanteral membrane	51
femur	48
tibiofemoral membrane	61
tibia	58
tibiotarsal membrane	48
pulvilli	31
Untreated	0

Necessary differences were 12.48 for $P = 0.05$; 16.80 for $P = 0.01$.

Lower mortality resulted from treatment of the pulvilli than from treatment of the other leg regions. Treatment of the tibiofemoral membrane produced

higher mortality than treatment of the tibiotarsal membrane and the femur; no significant difference was found between the tibiofemoral membrane, the tibia, and the coxotrochanteral membrane.

(*c*) *Coxotrochanteral Membrane and Coxal Membrane*

One μgm. of DDT was applied to each fly, and 80 flies (8 replicates of 10) were used for each site. After 24 hr. 56 flies treated on the coxal membrane and 61 of those treated on the coxotrochanteral membrane were dead. An analysis of variance showed no significant difference between these mortalities.

(*d*) *Foreleg*

The results show the comparative effectiveness of three areas of the foreleg as loci of application for DDT. Eighty flies (16 replicates of five flies each) were used for each treatment. After 24 hr. the number dead out of 80 flies treated with 1 μgm. DDT was as follows:

Treated on tibiofemoral membrane	53
coxal membrane	50
pretarsus	39
Untreated	0

Necessary differences were 11.53 for $P = 0.05$; 15.53 for $P = 0.01$.

These data show that the tibiofemoral membrane and the coxal membrane were more effective than the pretarsus as points of application for DDT.

(*e*) *Tibiofemoral Membrane of Foreleg, Midleg, and Hindleg*

One hundred flies (10 replicates of 10 flies each) were used for each locus. after 24 hr. the number dead was as follows:

Treated on the tibiofemoral membrane of foreleg	74
of midleg	58
of hindleg	54
Untreated	0

Necessary differences were 13.87 for $P = 0.05$; 18.99 for $P = 0.01$.

The tibiofemoral membrane of the foreleg was significantly more effective than that of the midleg, and very significantly more effective than that of the hindleg as a point of application for DDT.

(*f*) *Coxal Regions of Foreleg, Midleg, and Hindleg*

One hundred flies (10 replicates of 10 flies) were treated for each locus noted below. After 24 hr. the number dead, out of 100 flies treated with 1 μgm. DDT, was as follows:

Treated on coxal membrane of foreleg	82
coxal membrane of hindleg	65
coxotrochanteral membrane of midleg	47
Untreated	0

Necessary differences were: 10.84 for $P = 0.05$; 14.85 for $P = 0.01$.

These data indicate that DDT is very significantly more effective in the coxal region of the foreleg than in that of the midleg or the hindleg.

(*g*) *Tibiofemoral Membrane of the Foreleg and a Pulsatile Membrane in the Base of the Wing*

The results show a comparison of two sites containing pulsatile membranes (accessory hearts). One hundred and ten flies (11 replicates of 10 flies) were treated for each site. After 24 hr. the number dead out of 110 flies treated with 1 μgm. DDT was as follows:

Treated on tibiofemoral membrane	71
junction of cubitoanal and 1st anal cross vein	64
Untreated	0

Necessary differences were 20.9 for $P = 0.05$; 29.8 for $P = 0.01$.

There was no significant difference between these mortalities.

(*h*) *Head Regions*

One microgram of DDT was applied to each of four areas of the head: the pseudotracheae of the labella, the basal membrane of the antenna, the dorsal surface of the compound eye, and the dorsal side of the cervical membrane. Ten flies were used for each treatment area and the test was replicated 10 times. As preliminary tests gave complete mortality in 24 hr. for those treated on the labella, the observation time was reduced to 16 hr. After 16 hr. the number dead out of 100 flies treated with 1 μgm. DDT was as follows:

Treated on the labella	78
antennal membrane	62
compound eye	52
cervical membrane	47
Untreated	0

Necessary differences were 16.23 for $P = 0.05$; 21.92 for $P = 0.01$.

Treatment of the labella produced a higher mortality than treatment of the other head regions.

(*i*) *Speed of Knockdown in Relation to Locus of Application of DDT*

Sixty flies were treated on the labella with 1 μgm. DDT and released; 60 others were treated on the tibiofemoral membrane of the hindleg and similarly released. Results are shown in Table I.

TABLE I

NUMBER KNOCKED DOWN OUT OF 60 FLIES TREATED WITH 1 μGM. DDT

Time in hr.	0.5	1.0	2.0	3.0
Labella	53	51	48	48
Tibiofemoral membrane	0	0	41	41
Check	0	0	0	0

Knockdown was rapid in those flies treated on the labella but 5 out of 53 recovered completely. Those treated on the leg suffered no knockdown until after the first hour, but all those knocked down eventually died.

Relationship of Size of Treated Area to Effectiveness of DDT

The superiority of the labella as a locus for the application of DDT suggested that the size of the treated area may be important. The pseudotracheae of the labella may promote spreading of the applied droplet over a relatively larger area than would occur on cuticle at other loci. To test this possibility the cuticular area available to the DDT droplet was limited by cutting through the cuticle to isolate small squares from the remainder of the integument.

The dorsum of the mesothorax was chosen for the test site and "islands" of two sizes, the larger about four times the area of the smaller, were isolated in each of two lots of 100 flies. The special tape holder used in this test and the squares cut in the mesonotum of the fly are illustrated in Fig. 5. Haemolymph welling up through the incisions quickly coagulated and held the isolated squares of cuticle in place. A droplet containing 1 μgm. of DDT was placed in the center of each square.

In addition to the two lots of flies treated on the isolated "islands" of cuticle, 100 flies were treated on the intact mesonotum. After 20 hr. the number dead out of 100 flies treated with 1 μgm. DDT was as follows:

Treated on intact integument	67
large "island"	49
small "island"	29
Untreated	0

Necessary differences were 8.97 for $P = 0.05$; 10.29 for $P = 0.01$.

Mortalities were progressively smaller as the area available to the DDT was progressively limited; highly significant differences, in the direction of decreasing mortality were found between the intact integument, the large "island", and the small "island".

Topical Applications vs. Injections

These results suggested that the cuticle may play a more important role in the toxicity of DDT than merely to provide a route of entry into the organism. To test this possibility, 1 μgm. of DDT as a supersaturated droplet was applied to the mid-dorsal line of the mesonotum of 15 flies, and 15 other flies were treated with a 6.0 μgm. mass of crystalline DDT inserted into the muscles of the mesonotum through a flap cut in the cuticle. After 20 hr. five of those treated externally and two of those treated internally had died. None died in the check.

To increase the effective area of the injected DDT, the crystalline mass was left in a benzene–air atmosphere until a bloom of microscopic, sharp crystals projected from its surface. The external treatment dosage was doubled to

2.0 μgm. DDT per fly. Twenty flies were treated for each locus. After 24 hr. the number dead out of 20 flies treated with DDT was as follows:

2.0 μgm. applied externally on mesonotum as a supersaturated solution	16
6.0 μgm. DDT applied internally as a crystalline mass	1
Untreated	0

Hence, a large dosage of crystalline DDT inserted into the tissues of the mesonotum had no effect, but one-third of this dosage applied on the cuticle of the mesonotum produced a high mortality.

Influence of Quantity of Cuticular Waxes on Effectiveness of DDT

Ten milliliters of benzene was used to extract the cuticles of 100 male flies and DDT added to make a 1.07% DDT solution. Supersaturated droplets of this solution were then compared with the standard solution by applications to the tibiofemoral membranes on the hindlegs. One hundred flies were used for each treatment. A statistical analysis of the mortalities showed that the excess of wax neither increased nor decreased the effectiveness of DDT.

Discussion

This study affords a new approach to the problem of how flies exposed on a crystalline deposit of DDT acquire a lethal dose. Because the tarsus is the normal point of contact and because the walking position makes the pulvillus a good collecting surface (10), it has been assumed that the tarsomeres and particularly the pulvilli are indeed the normal sites of sorption. Yet this study shows that the pulvilli are relatively ineffective loci for the application of DDT, either as a crystalline mass or in a supersaturated benzene droplet; many other body areas were more effective points of application. It is suggested, therefore, that DDT accumulated on the tarsi is distributed subsequently to more susceptible areas by the cleaning movements of flies. These cleaning movements were invariably observed in nonimmobilized treated flies and were effected by the labella and legs. The labella themselves were shown in this study to be a highly effective point of application, and the cleaning movements with the legs would also serve to distribute the DDT to other sensitive loci.

Hayes and Liu (5) considered that the chemoreceptive sensilla found ventrally on the tarsomeres of the house fly leg might account for the effectiveness of DDT for that insect, while the lack of such sensilla on *Blatella germanica* L. adults and *Epilachna varivestis* Huls. larvae might be correlated with the resistance of those forms to DDT. The present study, however, shows that the first tarsomere which bears no sensilla (5) was a more effective point of application than the third, fourth, and fifth tarsomeres which have large numbers of sensilla. The claim that sensilla offer better entry points is based on the thinness of the articular membranes, but there is no evidence that the cuticle thickness in house flies plays any part in preventing or retarding DDT

penetration. Weismann (12) found resistant flies from Sweden had thicker cuticles than a susceptible strain from Switzerland, but this could have been due to natural variation in strains from different regions. March and Lewallen (7) could detect no significant difference in the cuticle thickness between susceptible and resistant fly strains.

Application of uniform doses of DDT to different loci produced widely different mortalities. The greatest difference was found between the pulvilli and the labella. Among these differences, there appears to be a tendency for the same dosage of DDT to produce lower mortalities as the locus of application moves distally along the legs, and posteriorly from head to abdomen. These gradients are most apparent when the extremes are considered; certain intermediate areas do not conform. For instance, significantly lower mortalities were obtained by treating the pulvillus than by treating the coxotrochanteral membrane; but the highest mortality was obtained on the intermediate tibiofemoral membrane. However, this membrane differs from the other loci in having beneath it a pulsatile organ which may assist the passage of DDT to its site of action. The base of the wing, where a pulsatile organ also occurs, was also a highly effective point of application. A more definite gradient of decreasing effectiveness was found as the locus of application was moved distally from the head areas, to the cervical membrane, to the coxal membrane of the foreleg, and to the coxal membrane of the hindleg. A similar gradient was found between the tibiofemoral membranes of the foreleg, midleg, and hindleg. Hockenyos and Lilly (6) reported an increasing gradient of effectiveness as the point of injection of nicotine into *Celerio lineata* larvae approached the head.

Sclerotization does not seem to influence the effectiveness of DDT applied in benzene solution; no difference in mortality was found between sclerotized areas of the legs (femur and tibia), and membranous areas without pulsatile organs (coxotrachanteral membrane and tibiotarsal membrane).

The study provided considerable evidence that the size of the cuticular area available to the DDT–benzene droplets has a profound effect on the resultant mortality. The effectiveness of the labella may have resulted from the relatively large area presented by the pseudotracheae; the pseudotracheae would tend to increase the spreading of the DDT droplet, by capillarity, over a comparatively large area of the integument. More convincing evidence for the importance of the size of cuticular area available to the DDT was obtained by the method of isolating "islands" of cuticle on the mesonotum. The progressive decrease in mortality obtained as the available area was decreased from intact integument, to large "island", to small "island" may be attributed to: the number of sensory endings affected; the amount of the cuticular nerve net exposed; the number of hypodermal cells affected; or simply the limit placed on the amount of DDT that can penetrate the cuticle in a given time.

Roeder and Weiant (9) found all sensory organs such as tactile setae to respond to DDT, so that differences in the numbers of these sense endings affected may be important.

A nerve net of anastomosing fibers is believed to exist in the insect integument. If this nerve net is associated with the toxic effect of DDT, then any limit on the portion of the net affected would be expected to reduce the mortality. This hypothesis supposes that DDT affects the peripheral nerves without reference to the central nervous system, as claimed by various authors (Roeder and Weiant (9), Witt (14), and Yeager and Munson (15)).

More recent evidence emphasises the role of the hypodermis in the toxic effect of DDT and lends some support to the idea that the number of hypodermal cells exposed to the DDT may be important.

Sternburg, Kearns, and Bruce (11) found that DDT-resistant flies metabolize DDT to the nontoxic compounds DDE (1, 1-dichloro-2,2-*bis* (*p*-chlorophenyl)-ethylene) and DDA (*bis*-(*p*-chlorophenyl)-acetic acid) in the cuticle hypoderm cells and in the digestive tract, and that this change is sufficiently rapid to account for their resistance. In susceptible flies, a small part of the DDT dosage was degraded to some other material, which may have been the actual toxin. Winteringham, Loveday, and Harrison (13) recognized this metabolism of DDT, but did not believe that it accounted for DDT resistance. They considered that DDT metabolism was an enzymic reaction and that the enzymic system involved is either destroyed or made inactive by the killing of the tissues.

If the portion of the DDT that kills the insect is thus altered by the hypodermal cells, the number of cells involved will be most important. It is conceivable that benzene or DDT might incapacitate cells immediately beneath the point of application where the concentration is highest so that only those cells lying beyond this region would prove effective in the toxic mechanism. To account for instances reported in the literature where direct application to isolated nerves produced symptoms, one must postulate a separate mechanism or suspect integumental contamination. Bodenstein (1) treated isolated portions of abdominal integument of *Drosophila viridis* with attached muscles, nerves, and tracheae, and produced symptoms in 30 sec. Though this suggests direct action on nerves, the hypodermis present may have produced toxic metabolites by enzymatic hydrolysis, and it is known that such hydrolyses can take place in a few milliseconds.

The finding that a large dosage of crystalline DDT placed directly into the tissues of the mesonotum had no toxic effect emphasizes again the importance of the cuticle. Various authors (Roeder and Weiant (8, 9), Bodenstein (1)) have shown that suspensions or emulsions of DDT injected into insects produce toxicity, but this finding does not exclude the possibility of contact with the hypodermis from within and alteration of the compound to a toxic form. If, then, the haemolymph is capable of transporting DDT in toxic quantities, the DDT must be presented as a suspension or emulsion or be altered by passage through the cuticle.

The observation that flies treated on the labella were knocked down more rapidly than those treated on the tibiofemoral membrane confirms the observation that the labella are particularly sensitive to DDT. In addition, flies

treated on the labella were observed to make vigorous cleaning movements immediately after treatments, and these movements would be expected to bring the DDT into contact with cuticular areas in addition to the highly sensitive labella. Flies treated on the tibiofemoral membrane did not begin cleaning movements for several minutes and, because of the observed rapidity of absorption, may not have spread the DDT far from the point of application.

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References

1. BODENSTEIN, D. Investigation on the locus of action of DDT in flies (*Drosophila*). Biol. Bull. 90 (2) : 148–157. 1946.
2. FISHER, R. W. and MORRISON, F. O. Methods of rearing and sexing (*Musca domestica* L.). 80th Ann. Rept. Entomol. Soc. Ontario, pp. 41–45. 1949.
3. GOODHUE, L. D. and LINNARD, C. E. Air separation apparatus for cleaning fly pupae. J. Econ. Entomol. 43 (2) : 228. 1950.
4. HAFEZ, M. A simple method for breeding the housefly, *Musca domestica* L. Bull. Entomol. Research, 39 (3) : 321–465. 1949.
5. HAYES, W. M. and YU-SU LIU. Tarsal chemoreceptors of the housefly and their possible relation to DDT toxicity. Ann. Entomol. Soc. Am. 40 : 401. 1947.
6. HOCKENYOS, G. and LILLY, T. Toxicity studies by hypodermic injection of *Celerio lineata* larvae. J. Econ. Entomol. 25 : 253. 1932.
7. MARCH, R. B. and LEWALLEN, L. L. A comparison of DDT-resistant and non-resistant house flies. J. Econ. Entomol. 43 (5) : 721–722. 1950.
8. ROEDER, K. D. and WEIANT, E. A. Site of action of DDT in the cockroach. Science, 103 : 304–306. 1946.
9. ROEDER, K. D. and WEIANT, E. A. The effect of DDT on sensory and motor structures in the cockroach leg. J. Cellular Comp. Physiol. 32 (2) : 175–186. 1948.
10. SARKARIA, D. S. and PATTON, R. L. Histological factors in the penetration of DDT through the pulvilli of several insect species. Trans. Am. Entomol. Soc. 75 (2) : 71–82. 1949.
11. STERNBURG, J., KEARNS, C. W., and BRUCE, W. N. Absorption and metabolism of DDT by resistant and susceptible houseflies. J. Econ. Entomol. 43 : 214–219. 1950.
12. WEISMANN, R. Untersuchungen uber das physiologische Verhalten von *Musca domestica* L. verschiedener provenienzen. Schweiz. Entomol. Gesell. Mitt. 20 (5) : 484–504. 1947.
13. WINTERINGHAM, F. P. W., LOVEDAY, P. M., and HARRISON, A. Resistance of houseflies to DDT. Nature, 167 (4238) : 106–107. 1951.
14. WITT, P. N. Ein Test zur Prufung der Wirksamkeit insekticider Substanzen und ein Beitrag zum Mechanismus der Wirkung von DDT und HCC. Z. Naturforsch. 2b (9/10) : 361–366. 1947.
15. YEAGER, J. F. and MUNSON, S. C. Physiological evidence of a site of action of DDT in an insect. Science, 102 : 305–307. 1945.

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Contents

Page

Occurrence of Amino Acids in the Honeydew of the Crescent-marked Lily Aphid, *Myzus circumflexus* (Buck.)—*J. B. Maltais* and *Jacques L. Auclair* - 191

Effects of Starvation on Larvae of the Pale Western Cutworm, *Agrotis orthogonia* Morr. (Lepidoptera : Phalaenidae)—*L. A. Jacobson* - - - - 194

Some Effects of Progesterone on the Response of the Immature Pullet to Estrogen, and Some Observations on the Effects of Gonadal Hormones on the Avian Spleen and Kidney—*W. E. J. Phillips, R. H. Common,* and *W. A. Maw* 201

The Aerobic Bacteria Associated with the Eastern Hemlock Looper, *Lambdina fiscellaria* (Gn.)—*T. A. Angus* - - - - - - - - - - 208

Factors Influencing the Performance of the Precipitin Test in the Determination of Blood Meals of Insects—*G. S. Eligh* - - - - - - - - - 213

The Bottom Sediments of Lake Lauzon, Montcalm County, Province of Quebec. I. Chemical and Physical Investigations—*Herman Kleerekoper* and *F. Grenier* - 219

Studies on the Biology of the Muskrat in Manitoba. Part I. Oestrous Cycle and Breeding Season—*J. A. McLeod* and *G. F. Bondar* - - - - - 243

The Importance of the Locus of Application on the Effectiveness of DDT for the House Fly, *Musca domestica* L. (Diptera : Muscidae)—*R. W. Fisher* - - 254